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<b>(21) International Application Number:</b> PCT/IB95/01167 <b>(22) International Filing Date:</b> 28 December 1995 (28.12.95)  <b>(30) Priority Data:</b> 08/366,779 30 December 1994 (30.12.94) US  <b>(71) Applicant:</b> RHONE-POULENC AGROCHIMIE [FR/FR]; 14-20, rue Pierre-Baizet, F-69263 Lyon (FR).  <b>(72) Inventors:</b> THOMAS, Terry, L.; 3004 Normand, College Station, TX 77845 (US). REDDY, Avum, S.; 3902 E. 29th Street #G11, Bryan, TX 77802 (US). NUCCIO, Michael; P.O. Box 553, College Station, TX 77841 (US). NUNBERG, Andrew, N.; 2804 B. Sprucewood Street, Bryan, TX 77801 (US). FREYSSINET, Georges, L.; 21, rue de Nervieux, F-69450 Saint-Cyr-au-Mont-d'Or (FR).  <b>(74) Agent:</b> MITSCHERLICH & PARTNER; Sonnenstrasse 33, D-80331 München (DE).		<b>(81) Designated States:</b> AU, BR, CA, CN, JP, RO, RU, UA, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).  <b>Published</b> <i>Without international search report and to be republished upon receipt of that report.</i>
<b>(54) Title:</b> PRODUCTION OF GAMMA LINOLENIC ACID BY A $\Delta 6$ -DESATURASE  <b>(57) Abstract</b>  Linoleic acid is converted into $\gamma$ -linolenic acid by the enzyme $\Delta 6$ -desaturase. The present invention is directed to isolated nucleic acids comprising the $\Delta 6$ -desaturase gene. More particularly, the isolated nucleic acid comprises the promoter, coding region and termination regions of the $\Delta 6$ -desaturase gene. The present invention provides recombinant constructions comprising the $\Delta 6$ -desaturase coding region in functional combination with heterologous regulatory sequences. The nucleic acids and recombinant constructions of the instant invention are useful in the production of GLA in transgenic organisms.		

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1 PRODUCTION OF GAMMA LINOLENIC ACID BY A  $\Delta 6$ -DESATURASE

Linoleic acid (18:2) (LA) is transformed into gamma linolenic acid (18:3) (GLA) by the enzyme  
5  $\Delta 6$ -desaturase. When this enzyme, or the nucleic acid encoding it, is transferred into LA-producing cells, GLA is produced. The present invention provides nucleic acids comprising the  $\Delta 6$ -desaturase gene. More specifically, the nucleic acids comprise the  
10 promoters, coding regions and termination regions of the  $\Delta 6$ -desaturase genes. The present invention is further directed to recombinant constructions comprising a  $\Delta 6$ -desaturase coding region in functional combination with heterologous regulatory sequences.  
15 The nucleic acids and recombinant constructions of the instant invention are useful in the production of GLA in transgenic organisms.

Unsaturated fatty acids such as linoleic ( $C_{18}\Delta^{9,12}$ ) and  $\alpha$ -linolenic ( $C_{18}\Delta^{9,12,15}$ ) acids are essential  
20 dietary constituents that cannot be synthesized by vertebrates since vertebrate cells can introduce double bonds at the  $\Delta^9$  position of fatty acids but cannot introduce additional double bonds between the  $\Delta^9$  double bond and the methyl-terminus of the fatty  
25 acid chain. Because they are precursors of other products, linoleic and  $\alpha$ -linolenic acids are essential fatty acids, and are usually obtained from plant sources. Linoleic acid can be converted by mammals into  $\gamma$ -linolenic acid (GLA,  $C_{18}\Delta^{6,9,12}$ ) which can in turn  
30 be converted to arachidonic acid (20:4), a critically

1 important fatty acid since it is an essential  
precursor of most prostaglandins.

The dietary provision of linoleic acid, by  
virtue of its resulting conversion to GLA and  
5 arachidonic acid, satisfies the dietary need for GLA  
and arachidonic acid. However, a relationship has  
been demonstrated between consumption of saturated  
fats and health risks such as hypercholesterolemia,  
atherosclerosis and other clinical disorders which  
10 correlate with susceptibility to coronary disease,  
while the consumption of unsaturated fats has been  
associated with decreased blood cholesterol  
concentration and reduced risk of atherosclerosis.  
The therapeutic benefits of dietary GLA may result  
15 from GLA being a precursor to arachidonic acid and  
thus subsequently contributing to prostaglandin  
synthesis. Accordingly, consumption of the more  
unsaturated GLA, rather than linoleic acid, has  
potential health benefits. However, GLA is not  
20 present in virtually any commercially grown crop  
plant.

Linoleic acid is converted into GLA by the  
enzyme  $\Delta 6$ -desaturase.  $\Delta 6$ -desaturase, an enzyme of  
more than 350 amino acids, has a membrane-bound domain  
25 and an active site for desaturation of fatty acids.  
When this enzyme is transferred into cells which  
endogenously produce linoleic acid but not GLA, GLA is  
produced. The present invention, by providing the  
gene encoding  $\Delta 6$ -desaturase, allows the production of  
30 transgenic organisms which contain functional  $\Delta 6$ -  
desaturase and which produce GLA. In addition to

1 allowing production of large amounts of GLA, the  
present invention provides new dietary sources of GLA.

The present invention is directed to  
isolated  $\Delta 6$ -desaturase genes. Specifically, the  
5 isolated genes comprises the  $\Delta 6$ -desaturase promoters,  
coding regions, and termination regions.

The present invention is further directed to  
expression vectors comprising the  $\Delta 6$ -desaturase  
promoter, coding region and termination region.

10 Yet another aspect of this invention is  
directed to expression vectors comprising a  $\Delta 6$ -  
desaturase coding region in functional combination  
with heterologous regulatory regions, i.e. elements  
not derived from the  $\Delta 6$ -desaturase gene.

15 Cells and organisms comprising the vectors  
of the present invention, and progeny of such  
organisms, are also provided by the present invention.

A further aspect of the present invention  
provides isolated bacterial  $\Delta 6$ -desaturase. An  
20 isolated plant  $\Delta 6$ -desaturase is also provided.

Yet another aspect of this invention  
provides a method for producing plants with increased  
gamma linolenic acid content.

A method for producing chilling tolerant  
25 plants is also provided by the present invention.

Fig. 1 depicts the hydropathy profiles of  
the deduced amino acid sequences of Synechocystis  $\Delta 6$ -  
desaturase (Panel A) and  $\Delta 12$ -desaturase (Panel B).  
Putative membrane spanning regions are indicated by  
30 solid bars. Hydrophobic index was calculated for a

1 window size of 19 amino acid residues [Kyte, et al.  
(1982) J. Molec. Biol. 157].

Fig. 2 provides gas liquid chromatography  
profiles of wild type (Panel A) and transgenic (Panel  
5 B) Anabaena.

Fig. 3 is a diagram of maps of cosmid cSy75,  
cSy13 and Csy7 with overlapping regions and subclones.  
The origins of subclones of Csy75, Csy75-3.5 and Csy7  
are indicated by the dashed diagonal lines.  
10 Restriction sites that have been inactivated are in  
parentheses.

Fig. 4 provides gas liquid chromatography  
profiles of wild type (Panel A) and transgenic (Panel  
B) tobacco.

15 Fig. 5A depicts the DNA sequence of a  $\Delta$ -6  
desaturase cDNA isolated from borage.

Fig. 5B depicts the protein sequence of the  
open reading frame in the isolated borage  $\Delta$ -6  
desaturase cDNA. Three amino acid motifs  
20 characteristic of desaturases are indicated and are,  
in order, lipid box, metal box 1, and metal box 2.

Fig. 6 is a dendrogram showing similarity of  
the borage  $\Delta$ 6-desaturase to other membrane-bound  
desaturases. The amino acid sequence of the borage  
25  $\Delta$ 6-desaturase was compared to other known desaturases  
using Gene Works (IntelliGenetics). Numerical values  
correlate to relative phylogenetic distances between  
subgroups compared.

Fig. 7 is a restriction map of 221. $\Delta$ 6.NOS  
30 and 121. $\Delta$ 6.NOS. In 221. $\Delta$ 6.NOS, the remaining portion

1 of the plasmid is pBI221 and in 121.Δ6.NOS, the  
remaining portion of the plasmid is pBI121.

Fig. 8 provides gas liquid chromatography  
profiles of mock transfected (Panel A) and 221.Δ6.NOS  
5 transfected (Panel B) carrot cells. The positions of  
18:2, 18:3 α, and 18:3 γ (GLA) are indicated.

Fig. 9 provides gas liquid chromatography  
profiles of an untransformed tobacco leaf (Panel A)  
and a tobacco leaf transformed with 121.Δ6.NOS. The  
10 positions of 18:2, 18:3 α, 18:3γ (GLA), and 18:4 are  
indicated.

Fig. 10 provides gas liquid chromatography  
profiles for untransformed tobacco seeds (Panel A) and  
seeds of tobacco transformed with 121.Δ6.NOS. The  
15 positions of 18:2, 18:3α and 18:3γ (GLA) are indicated.

The present invention provides isolated  
nucleic acids encoding Δ6-desaturase. To identify a  
nucleic acid encoding Δ6-desaturase, DNA is isolated  
from an organism which produces GLA. Said organism  
20 can be, for example, an animal cell, certain fungi  
(e.g. Mortierella), certain bacteria (e.g.  
Synechocystis) or certain plants (borage, Oenothera,  
currants). The isolation of genomic DNA can be  
accomplished by a variety of methods well-known to one  
25 of ordinary skill in the art, as exemplified by  
Sambrook et al. (1989) in Molecular Cloning: A  
Laboratory Manual, Cold Spring Harbor, NY. The  
isolated DNA is fragmented by physical methods or  
enzymatic digestion and cloned into an appropriate  
30 vector, e.g. a bacteriophage or cosmid vector, by any  
of a variety of well-known methods which can be found

1 in references such as Sambrook et al. (1989).  
Expression vectors containing the DNA of the present  
invention are specifically contemplated herein. DNA  
encoding  $\Delta 6$ -desaturase can be identified by gain of  
5 function analysis. The vector containing fragmented  
DNA is transferred, for example by infection,  
transconjugation, transfection, into a host organism  
that produces linoleic acid but not GLA. As used  
herein, "transformation" refers generally to the  
10 incorporation of foreign DNA into a host cell.  
Methods for introducing recombinant DNA into a host  
organism are known to one of ordinary skill in the art  
and can be found, for example, in Sambrook et al.  
(1989). Production of GLA by these organisms (i.e.,  
15 gain of function) is assayed, for example by gas  
chromatography or other methods known to the  
ordinarily skilled artisan. Organisms which are  
induced to produce GLA, i.e. have gained function by  
the introduction of the vector, are identified as  
20 expressing DNA encoding  $\Delta 6$ -desaturase, and said DNA is  
recovered from the organisms. The recovered DNA can  
again be fragmented, cloned with expression vectors,  
and functionally assessed by the above procedures to  
define with more particularity the DNA encoding  $\Delta 6$ -  
25 desaturase.

As an example of the present invention,  
random DNA is isolated from the cyanobacteria  
Synechocystis Pasteur Culture Collection (PCC) 6803,  
American Type Culture Collection (ATCC) 27184, cloned  
30 into a cosmid vector, and introduced by  
transconjugation into the GLA-deficient cyanobacterium



1 Anabaena strain PCC 7120, ATCC 27893. Production of GLA from Anabaena linoleic acid is monitored by gas chromatography and the corresponding DNA fragment is isolated.

5               The isolated DNA is sequenced by methods well-known to one of ordinary skill in the art as found, for example, in Sambrook et al. (1989).

              In accordance with the present invention, DNA molecules comprising  $\Delta 6$ -desaturase genes have been  
10 isolated. More particularly, a 3.588 kilobase (kb) DNA comprising a  $\Delta 6$ -desaturase gene has been isolated from the cyanobacteria Synechocystis. The nucleotide sequence of the 3.588 kb DNA was determined and is shown in SEQ ID NO:1. Open reading frames defining  
15 potential coding regions are present from nucleotide 317 to 1507 and from nucleotide 2002 to 3081. To define the nucleotides responsible for encoding  $\Delta 6$ -desaturase, the 3.588 kb fragment that confers  $\Delta 6$ -desaturase activity is cleaved into two subfragments,  
20 each of which contains only one open reading frame. Fragment ORF1 contains nucleotides 1 through 1704, while fragment ORF2 contains nucleotides 1705 through 3588. Each fragment is subcloned in both forward and reverse orientations into a conjugal expression vector  
25 (AM542, Wolk et al. [1984] Proc. Natl. Acad. Sci. USA 81, 1561) that contains a cyanobacterial carboxylase promoter. The resulting constructs (i.e. ORF1(F), ORF1(R), ORF2(F) and ORF2(R)) are conjugated to wild-type Anabaena PCC 7120 by standard methods (see, for  
30 example, Wolk et al. (1984) Proc. Natl. Acad. Sci. USA 81, 1561). Conjugated cells of Anabaena are

1 identified as Neo<sup>R</sup> green colonies on a brown  
background of dying non-conjugated cells after two  
weeks of growth on selective media (standard mineral  
media BG11N + containing 30µg/ml of neomycin according  
5 to Rippka et al., (1979) J. Gen Microbiol. 111, 1).  
The green colonies are selected and grown in selective  
liquid media (BG11N + with 15µg/ml neomycin). Lipids  
are extracted by standard methods (e.g. Dahmer et al.,  
(1989) Journal of American Oil Chemical Society 66,  
10 543) from the resulting transconjugants containing the  
forward and reverse oriented ORF1 and ORF2 constructs.  
For comparison, lipids are also extracted from wild-  
type cultures of Anabaena and Synechocystis. The  
fatty acid methyl esters are analyzed by gas liquid  
15 chromatography (GLC), for example with a Tracor-560  
gas liquid chromatograph equipped with a hydrogen  
flame ionization detector and a capillary column. The  
results of GLC analysis are shown in Table 1.

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1 Table 1: Occurrence of C18 fatty acids in wild-type  
and transgenic cyanobacteria

	SOURCE	18:0	18:1	18:2	$\gamma$ 18:3	$\alpha$ 18:3	18:4
5	Anabaena (wild type)	+	+	+	-	+	-
	Anabaena + ORF1(F)	+	+	+	-	+	-
	Anabaena + ORF1(R)	+	+	+	-	+	-
	Anabaena + ORF2(F)	+	+	+	+	+	+
10	Anabaena + ORF2(R)	+	+	+	-	+	-
	Synechocystis (wild type)	+	+	+	+	-	-

As assessed by GLC analysis, GLA deficient  
 15 Anabaena gain the function of GLA production when the  
 construct containing ORF2 in forward orientation is  
 introduced by transconjugation. Transconjugants  
 containing constructs with ORF2 in reverse orientation  
 to the carboxylase promoter, or ORF1 in either  
 20 orientation, show no GLA production. This analysis  
 demonstrates that the single open reading frame (ORF2)  
 within the 1884 bp fragment encodes  $\Delta$ 6-desaturase.  
 The 1884 bp fragment is shown as SEQ ID NO:3. This is  
 substantiated by the overall similarity of the  
 25 hydropathy profiles between  $\Delta$ 6-desaturase and  $\Delta$ 12-  
 desaturase [Wada *et al.* (1990) Nature 347] as shown in  
 Fig. 1 as (A) and (B), respectively.

Also in accordance with the present  
 invention, a cDNA comprising a  $\Delta$ 6-desaturase gene from  
 30 borage (Borago officinalis) has been isolated. The  
 nucleotide sequence of the 1.685 kilobase (kb) cDNA

1 was determined and is shown in Fig. 5A (SEQ ID NO: 4).  
The ATG start codon and stop codon are underlined.  
The amino acid sequence corresponding to the open  
reading frame in the borage delta 6-desaturase is  
5 shown in Fig. 5B (SEQ ID NO: 5).

Isolated nucleic acids encoding  $\Delta 6$ -  
desaturase can be identified from other GLA-producing  
organisms by the gain of function analysis described  
above, or by nucleic acid hybridization techniques  
10 using the isolated nucleic acid which encodes  
Synechocystis or borage  $\Delta 6$ -desaturase as a  
hybridization probe. Both genomic and cDNA cloning  
methods are known to the skilled artisan and are  
contemplated by the present invention. The  
15 hybridization probe can comprise the entire DNA  
sequence disclosed as SEQ. ID NO:1 or SEQ. ID NO:4, or  
a restriction fragment or other DNA fragment thereof,  
including an oligonucleotide probe. Methods for  
cloning homologous genes by cross-hybridization are  
20 known to the ordinarily skilled artisan and can be  
found, for example, in Sambrook (1989) and Beltz et  
al. (1983) Methods in Enzymology 100, 266.

In another method of identifying a delta 6-  
desaturase gene from an organism producing GLA, a cDNA  
25 library is made from poly-A<sup>+</sup> RNA isolated from  
polysomal RNA. In order to eliminate hyper-abundant  
expressed genes from the cDNA population, cDNAs or  
fragments thereof corresponding to hyper-abundant  
cDNAs genes are used as hybridization probes to the  
30 cDNA library. Non hybridizing plaques are excised and  
the resulting bacterial colonies are used to inoculate

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1 liquid cultures and sequenced. For example, as a  
means of eliminating other seed storage protein cDNAs  
from a cDNA library made from borage polysomal RNA,  
cDNAs corresponding to abundantly expressed seed  
5 storage proteins are first hybridized to the cDNA  
library. The "subtracted" DNA library is then used to  
generate expressed sequence tags (ESTs) and such tags  
are used to scan a data base such as GenBank to  
identify potential desaturates.

10 Transgenic organisms which gain the function  
of GLA production by introduction of DNA encoding  $\Delta$ -  
desaturase also gain the function of  
octadecatetraeonic acid ( $18:4^{\Delta 6,9,12,15}$ ) production.  
Octadecatetraeonic acid is present normally in fish  
15 oils and in some plant species of the Boraginaceae  
family (Craig et al. [1964] J. Amer. Oil Chem. Soc.  
41, 209-211; Gross et al. [1976] Can. J. Plant Sci.  
56, 659-664). In the transgenic organisms of the  
present invention, octadecatetraenoic acid results  
20 from further desaturation of  $\alpha$ -linolenic acid by  $\Delta 6$ -  
desaturase or desaturation of GLA by  $\Delta 15$ -desaturase.

The 359 amino acids encoded by ORF2, i.e.  
the open reading frame encoding Synechocystis  $\Delta 6$ -  
desaturase, are shown as SEQ. ID NO:2. The open  
25 reading frame encoding the borage  $\Delta 6$ -desaturase is  
shown in SEQ ID NO: 5. The present invention further  
contemplates other nucleotide sequences which encode  
the amino acids of SEQ ID NO:2 and SEQ ID NO: 5. It  
is within the ken of the ordinarily skilled artisan to  
30 identify such sequences which result, for example,  
from the degeneracy of the genetic code. Furthermore,

1 one of ordinary skill in the art can determine, by the  
gain of function analysis described hereinabove,  
smaller subfragments of the fragments containing the  
open reading frames which encode  $\Delta 6$ -desaturases.

5 The present invention contemplates any such  
polypeptide fragment of  $\Delta 6$ -desaturase and the nucleic  
acids therefor which retain activity for converting LA  
to GLA.

In another aspect of the present invention,  
10 a vector containing a nucleic acid of the present  
invention or a smaller fragment containing the  
promoter, coding sequence and termination region of a  
 $\Delta 6$ -desaturase gene is transferred into an organism,  
for example, cyanobacteria, in which the  $\Delta 6$ -desaturase  
15 promoter and termination regions are functional.  
Accordingly, organisms producing recombinant  $\Delta 6$ -  
desaturase are provided by this invention. Yet  
another aspect of this invention provides isolated  $\Delta 6$ -  
desaturase, which can be purified from the recombinant  
20 organisms by standard methods of protein purification.  
(For example, see Ausubel et al. [1987] Current  
Protocols in Molecular Biology, Green Publishing  
Associates, New York).

Vectors containing DNA encoding  $\Delta 6$ -  
25 desaturase are also provided by the present invention.  
It will be apparent to one of ordinary skill in the  
art that appropriate vectors can be constructed to  
direct the expression of the  $\Delta 6$ -desaturase coding  
sequence in a variety of organisms. Replicable  
30 expression vectors are particularly preferred.  
Replicable expression vectors as described herein are

1 DNA or RNA molecules engineered for controlled  
expression of a desired gene, i.e. the  $\Delta 6$ -desaturase  
gene. Preferably the vectors are plasmids,  
bacteriophages, cosmids or viruses. Shuttle vectors,  
5 e.g. as described by Wolk et al. (1984) Proc. Natl.  
Acad. Sci. USA, 1561-1565 and Bustos et al. (1991) J.  
Bacteriol. 174, 7525-7533, are also contemplated in  
accordance with the present invention. Sambrook et  
al. (1989), Goeddel, ed. (1990) Methods in Enzymology  
10 185 Academic Press, and Perbal (1988) A Practical  
Guide to Molecular Cloning, John Wiley and Sons, Inc.,  
provide detailed reviews of vectors into which a  
nucleic acid encoding the present  $\Delta 6$ -desaturase can be  
inserted and expressed. Such vectors also contain  
15 nucleic acid sequences which can effect expression of  
nucleic acids encoding  $\Delta 6$ -desaturase. Sequence  
elements capable of effecting expression of a gene  
product include promoters, enhancer elements, upstream  
activating sequences, transcription termination  
20 signals and polyadenylation sites. Both constitutive  
and tissue specific promoters are contemplated. For  
transformation of plant cells, the cauliflower mosaic  
virus (CaMV) 35S promoter and promoters which are  
regulated during plant seed maturation are of  
25 particular interest. All such promoter and  
transcriptional regulatory elements, singly or in  
combination, are contemplated for use in the present  
replicable expression vectors and are known to one of  
ordinary skill in the art. The CaMV 35S promoter is  
30 described, for example, by Restrepo et al. (1990)

1 Plant Cell 2, 987. Genetically engineered and mutated  
regulatory sequences are also contemplated.

The ordinarily skilled artisan can determine  
vectors and regulatory elements suitable for  
5 expression in a particular host cell. For example, a  
vector comprising the promoter from the gene encoding  
the carboxylase of Anabaena operably linked to the  
coding region of  $\Delta 6$ -desaturase and further operably  
linked to a termination signal from Synechocystis is  
10 appropriate for expression of  $\Delta 6$ -desaturase in  
cyanobacteria. "Operably linked" in this context  
means that the promoter and terminator sequences  
effectively function to regulate transcription. As a  
further example, a vector appropriate for expression  
15 of  $\Delta 6$ -desaturase in transgenic plants can comprise a  
seed-specific promoter sequence derived from  
helianthinin, napin, or glycinin operably linked to  
the  $\Delta 6$ -desaturase coding region and further operably  
linked to a seed termination signal or the nopaline  
20 synthase termination signal. As a still further  
example, a vector for use in expression of  $\Delta 6$ -  
desaturase in plants can comprise a constitutive  
promoter or a tissue specific promoter operably linked  
to the  $\Delta 6$ -desaturase coding region and further  
25 operably linked to a constitutive or tissue specific  
terminator or the nopaline synthase termination  
signal.

In particular, the helianthinin regulatory  
elements disclosed in applicant's copending U.S.  
30 Application Serial No. 682,354, filed April 8, 1991  
and incorporated herein by reference, are contemplated



1 as promoter elements to direct the expression of the  
Δ6-desaturase of the present invention.

Modifications of the nucleotide sequences or  
regulatory elements disclosed herein which maintain  
5 the functions contemplated herein are within the scope  
of this invention. Such modifications include  
insertions, substitutions and deletions, and  
specifically substitutions which reflect the  
degeneracy of the genetic code.

10 Standard techniques for the construction of  
such hybrid vectors are well-known to those of  
ordinary skill in the art and can be found in  
references such as Sambrook *et al.* (1989), or any of  
the myriad of laboratory manuals on recombinant DNA  
15 technology that are widely available. A variety of  
strategies are available for ligating fragments of  
DNA, the choice of which depends on the nature of the  
termini of the DNA fragments. It is further  
contemplated in accordance with the present invention  
20 to include in the hybrid vectors other nucleotide  
sequence elements which facilitate cloning, expression  
or processing, for example sequences encoding signal  
peptides, a sequence encoding KDEL, which is required  
for retention of proteins in the endoplasmic reticulum  
25 or sequences encoding transit peptides which direct  
Δ6-desaturase to the chloroplast. Such sequences are  
known to one of ordinary skill in the art. An  
optimized transit peptide is described, for example,  
by Van den Broeck *et al.* (1985) *Nature* 313, 358.  
30 Prokaryotic and eukaryotic signal sequences are

1 disclosed, for example, by Michaelis et al. (1982)  
Ann. Rev. Microbiol. 36, 425.

A further aspect of the instant invention provides organisms other than cyanobacteria or plants  
5 which contain the DNA encoding the  $\Delta 6$ -desaturase of the present invention. The transgenic organisms contemplated in accordance with the present invention include bacteria, cyanobacteria, fungi, and plants and animals. The isolated DNA of the present invention  
10 can be introduced into the host by methods known in the art, for example infection, transfection, transformation or transconjugation. Techniques for transferring the DNA of the present invention into such organisms are widely known and provided in  
15 references such as Sambrook et al. (1989).

A variety of plant transformation methods are known. The  $\Delta 6$ -desaturase gene can be introduced into plants by a leaf disk transformation-regeneration procedure as described by Horsch et al. (1985) Science  
20 227, 1229. Other methods of transformation, such as protoplast culture (Horsch et al. (1984) Science 223, 496; DeBlock et al. (1984) EMBO J. 2, 2143; Barton et al. (1983) Cell 32, 1033) can also be used and are within the scope of this invention. In a preferred  
25 embodiment plants are transformed with Agrobacterium-derived vectors. However, other methods are available to insert the  $\Delta 6$ -desaturase genes of the present invention into plant cells. Such alternative methods include biolistic approaches (Klein et al. (1987)  
30 Nature 327, 70), electroporation, chemically-induced DNA uptake, and use of viruses or pollen as vectors.

1                   When necessary for the transformation  
method, the  $\Delta 6$ -desaturase genes of the present  
invention can be inserted into a plant transformation  
vector, e.g. the binary vector described by Bevan  
5 (1984) Nucleic Acids Res. 12, 8111. Plant  
transformation vectors can be derived by modifying the  
natural gene transfer system of Agrobacterium  
tumefaciens. The natural system comprises large Ti  
(tumor-inducing)-plasmids containing a large segment,  
10 known as T-DNA, which is transferred to transformed  
plants. Another segment of the Ti plasmid, the vir  
region, is responsible for T-DNA transfer. The T-DNA  
region is bordered by terminal repeats. In the  
modified binary vectors the tumor-inducing genes have  
15 been deleted and the functions of the vir region are  
utilized to transfer foreign DNA bordered by the T-DNA  
border sequences. The T-region also contains a  
selectable marker for antibiotic resistance, and a  
multiple cloning site for inserting sequences for  
20 transfer. Such engineered strains are known as  
"disarmed" A. tumefaciens strains, and allow the  
efficient transformation of sequences bordered by the  
T-region into the nuclear genomes of plants.

                  Surface-sterilized leaf disks are inoculated  
25 with the "disarmed" foreign DNA-containing A.  
tumefaciens, cultured for two days, and then  
transferred to antibiotic-containing medium.  
Transformed shoots are selected after rooting in  
medium containing the appropriate antibiotic,  
30 transferred to soil and regenerated.

1           Another aspect of the present invention  
provides transgenic plants or progeny of these plants  
containing the isolated DNA of the invention. Both  
monocotyledenous and dicotyledenous plants are  
5 contemplated. Plant cells are transformed with the  
isolated DNA encoding  $\Delta 6$ -desaturase by any of the  
plant transformation methods described above. The  
transformed plant cell, usually in a callus culture or  
leaf disk, is regenerated into a complete transgenic  
10 plant by methods well-known to one of ordinary skill  
in the art (e.g. Horsch *et al.* (1985) *Science* 227,  
1129). In a preferred embodiment, the transgenic  
plant is sunflower, oil seed rape, maize, tobacco,  
peanut or soybean. Since progeny of transformed  
15 plants inherit the DNA encoding  $\Delta 6$ -desaturase, seeds  
or cuttings from transformed plants are used to  
maintain the transgenic plant line.

The present invention further provides a  
method for providing transgenic plants with an  
20 increased content of GLA. This method includes  
introducing DNA encoding  $\Delta 6$ -desaturase into plant  
cells which lack or have low levels of GLA but contain  
LA, and regenerating plants with increased GLA content  
from the transgenic cells. In particular,  
25 commercially grown crop plants are contemplated as the  
transgenic organism, including, but not limited to,  
sunflower, soybean, oil seed rape, maize, peanut and  
tobacco.

The present invention further provides a  
30 method for providing transgenic organisms which  
contain GLA. This method comprises introducing DNA

1 encoding  $\Delta 6$ -desaturase into an organism which lacks or  
has low levels of GLA, but contains LA. In another  
embodiment, the method comprises introducing one or  
more expression vectors which comprise DNA encoding  
5  $\Delta 12$ -desaturase and  $\Delta 6$ -desaturase into organisms which  
are deficient in both GLA and LA. Accordingly,  
organisms deficient in both LA and GLA are induced to  
produce LA by the expression of  $\Delta 12$ -desaturase, and  
GLA is then generated due to the expression of  $\Delta 6$ -  
10 desaturase. Expression vectors comprising DNA  
encoding  $\Delta 12$ -desaturase, or  $\Delta 12$ -desaturase and  $\Delta 6$ -  
desaturase, can be constructed by methods of  
recombinant technology known to one of ordinary skill  
in the art (Sambrook et al., 1989) and the published  
15 sequence of  $\Delta 12$ -desaturase (Wada et al. [1990] Nature  
(London) 347, 200-203. In addition, it has been  
discovered in accordance with the present invention  
that nucleotides 2002-3081 of SEQ. ID NO:1 encode  
cyanobacterial  $\Delta 12$ -desaturase. Accordingly, this  
20 sequence can be used to construct the subject  
expression vectors. In particular, commercially grown  
crop plants are contemplated as the transgenic  
organism, including, but not limited to, sunflower,  
soybean, oil seed rape, maize, peanut and tobacco.

25 The present invention is further directed to  
a method of inducing chilling tolerance in plants.  
Chilling sensitivity may be due to phase transition of  
lipids in cell membranes. Phase transition  
temperature depends upon the degree of unsaturation of  
fatty acids in membrane lipids, and thus increasing  
30 the degree of unsaturation, for example by introducing

1  $\Delta$ 6-desaturase to convert LA to GLA, can induce or  
improve chilling resistance. Accordingly, the present  
method comprises introducing DNA encoding  $\Delta$ 6-  
desaturase into a plant cell, and regenerating a plant  
5 with improved chilling resistance from said  
transformed plant cell. In a preferred embodiment,  
the plant is a sunflower, soybean, oil seed rape,  
maize, peanut or tobacco plant.

The following examples further illustrate  
10 the present invention.

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## EXAMPLE 1

## Strains and Culture Conditions

Synechocystis (PCC 6803, ATCC 27184),  
5 Anabaena (PCC 7120, ATCC 27893) and Synechococcus (PCC  
7942, ATCC 33912) were grown photoautotrophically at  
30°C in BG11N+ medium (Rippka et al. [1979] J. Gen.  
Microbiol. 111, 1-61) under illumination of  
incandescent lamps  
10 ( $60\mu\text{E}\cdot\text{m}^{-2}\cdot\text{S}^{-1}$ ). Cosmids and plasmids were selected and  
propagated in Escherichia coli strain DH5 $\alpha$  on LB  
medium supplemented with antibiotics at standard  
concentrations as described by Maniatis et al. (1982)  
Molecular Cloning: A Laboratory Manual, Cold Spring  
15 Harbor Laboratory, Cold Spring, New York.

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## EXAMPLE 2

Construction of Synechocystis Cosmid Genomic Library

5 Total genomic DNA from Synechocystis (PCC 6803) was partially digested with Sau3A and fractionated on a sucrose gradient (Ausubel et al. [1987] Current Protocols in Molecular Biology, Greene Publishing Associates and Wiley Interscience, New York). Fractions containing 30 to 40 kb DNA fragments  
10 were selected and ligated into the dephosphorylated BamHI site of the cosmid vector, pDUCA7 (Buikema et al. [1991] J. Bacteriol. 173, 1879-1885). The ligated DNA was packaged in vitro as described by Ausubel et al. (1987), and packaged phage were propagated in E.  
15 coli DH5 $\alpha$  containing the AvaI and Eco4711 methylase helper plasmid, pRL528 as described by Buikema et al. (1991). A total of 1152 colonies were isolated randomly and maintained individually in twelve 96-well microtiter plates.

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## EXAMPLE 3

Gain-of-Function Expression of GLA in Anabaena

Anabaena (PCC 7120), a filamentous  
5 cyanobacterium, is deficient in GLA but contains  
significant amounts of linoleic acid, the precursor  
for GLA (Figure 2; Table 2). The Synechocystis cosmid  
library described in Example 2 was conjugated into  
10 Anabaena (PCC 7120) to identify transconjugants that  
produce GLA. Anabaena cells were grown to mid-log  
phase in BG11N+ liquid medium and resuspended in the  
same medium to a final concentration of approximately  
2x10<sup>8</sup> cells per ml. A mid-log phase culture of E.  
15 coli RP4 (Burkardt et al. [1979] J. Gen. Microbiol.  
114, 341-348) grown in LB containing ampicillin was  
washed and resuspended in fresh LB medium. Anabaena  
and RP4 were then mixed and spread evenly on BG11N+  
plates containing 5% LB. The cosmid genomic library  
20 was replica plated onto LB plates containing 50 µg/ml  
kanamycin and 17.5 µg/ml chloramphenicol and was  
subsequently patched onto BG11N+ plates containing  
Anabaena and RP4. After 24 hours of incubation at  
30°C, 30 µg/ml of neomycin was underlaid; and  
incubation at 30°C was continued until transconjugants  
25 appeared.

Individual transconjugants were isolated  
after conjugation and grown in 2 ml BG11N+ liquid  
medium with 15 µg/ml neomycin. Fatty acid methyl  
esters were prepared from wild type cultures and  
30 cultures containing pools of ten transconjugants as  
follows. Wild type and transgenic cyanobacterial

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1 cultures were harvested by centrifugation and washed  
twice with distilled water. Fatty acid methyl esters  
were extracted from these cultures as described by  
Dahmer et al. (1989) J. Amer. Oil. Chem. Soc. 66, 543-  
5 548 and were analyzed by Gas Liquid Chromatography  
(GLC) using a Tracor-560 equipped with a hydrogen  
flame ionization detector and capillary column (30 m x  
0.25 mm bonded FSOT Superox II, Alltech Associates  
Inc., IL). Retention times and co-chromatography of  
10 standards (obtained from Sigma Chemical Co.) were used  
for identification of fatty acids. The average fatty  
acid composition was determined as the ratio of peak  
area of each C18 fatty acid normalized to an internal  
standard.

15 Representative GLC profiles are shown in  
Fig. 2. C18 fatty acid methyl esters are shown.  
Peaks were identified by comparing the elution times  
with known standards of fatty acid methyl esters and  
were confirmed by gas chromatography-mass  
20 spectrometry. Panel A depicts GLC analysis of fatty  
acids of wild type Anabaena. The arrow indicates the  
migration time of GLA. Panel B is a GLC profile of  
fatty acids of transconjugants of Anabaena with  
pAM542+1.8F. Two GLA producing pools (of 25 pools  
25 representing 250 transconjugants) were identified that  
produced GLA. Individual transconjugants of each GLA  
positive pool were analyzed for GLA production; two  
independent transconjugants, AS13 and AS75, one from  
each pool, were identified which expressed significant  
30 levels of GLA and which contained cosmids, cSy13 and  
cSy75, respectively (Figure 3). The cosmids overlap

1 in a region approximately 7.5 kb in length. A 3.5 kb  
NheI fragment of cSy75 was recloned in the vector  
pDUCA7 and transferred to Anabaena resulting in gain-  
of-function expression of GLA (Table 2).

5 Two NheI/Hind III subfragments (1.8 and 1.7  
kb) of the 3.5 kb Nhe I fragment of cSy75-3.5 were  
subcloned into "pBLUESCRIPT" (Stratagene) (Figure 3)  
for sequencing. Standard molecular biology techniques  
were performed as described by Maniatis et al. (1982)  
10 and Ausubel et al. (1987). Dideoxy sequencing (Sanger  
et al. [1977] Proc. Natl. Acad. Sci. USA 74, 5463-  
5467) of pBS1.8 was performed with "SEQUENASE" (United  
States Biochemical) on both strands by using specific  
oligonucleotide primers synthesized by the Advanced  
15 DNA Technologies Laboratory (Biology Department, Texas  
A & M University). DNA sequence analysis was done  
with the GCG (Madison, WI) software as described by  
Devereux et al. (1984) Nucleic Acids Res. 12, 387-395.

Both NheI/HindIII subfragments were  
20 transferred into a conjugal expression vector, AM542,  
in both forward and reverse orientations with respect  
to a cyanobacterial carboxylase promoter and were  
introduced into Anabaena by conjugation.  
Transconjugants containing the 1.8 kb fragment in the  
25 forward orientation (AM542-1.8F) produced significant  
quantities of GLA and octadecatetraenoic acid (Figure  
2; Table 2). Transconjugants containing other  
constructs, either reverse oriented 1.8 kb fragment or  
forward and reverse oriented 1.7 kb fragment, did not  
30 produce detectable levels of GLA (Table 2).

1           Figure 2 compares the C18 fatty acid profile  
of an extract from wild type Anabaena (Figure 2A) with  
that of transgenic Anabaena containing the 1.8 kb  
fragment of cSy75-3.5 in the forward orientation  
5 (Figure 2B). GLC analysis of fatty acid methyl esters  
from AM542-1.8F revealed a peak with a retention time  
identical to that of authentic GLA standard. Analysis  
of this peak by gas chromatography-mass spectrometry  
(GC-MS) confirmed that it had the same mass  
10 fragmentation pattern as a GLA reference sample.  
Transgenic Anabaena with altered levels of  
polyunsaturated fatty acids were similar to wild type  
in growth rate and morphology.

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1 Table 2 Composition of C18 Fatty Acids in Wild Type  
and Transgenic Cyanobacteria

		Fatty Acid (%)					
Strain		18:0	18:1	18:2	18:3(α)	18:3(γ)	18:4
Wild Type							
5	<i>Synechocystis</i> (sp.PCC6803)	13.6	4.5	54.5	-	27.3	-
10	<i>Anabaena</i> (sp.PCC7120)	2.9	24.8	37.1	35.2	-	-
15	<i>Synechococcus</i> (sp.PCC7942)	20.6	79.4	-	-	-	-
Anabaena Transconjugants							
	cSy75	3.8	24.4	22.3	9.1	27.9	12.5
	cSy75-3.5	4.3	27.6	18.1	3.2	40.4	6.4
20	pAM542 - 1.8P	4.2	13.9	12.1	19.1	25.4	25.4
	pAM542 - 1.8R	7.7	23.1	38.4	30.8	-	-
	pAM542 - 1.7P	2.8	27.8	36.1	33.3	-	-
	pAM542 - 1.7R	2.8	25.4	42.3	29.6	-	-
Synechococcus Transformants							
25	pAM854	27.8	72.2	-	-	-	-
	pAM854 -Δ <sup>12</sup>	4.0	43.2	46.0	-	-	-
	pAM854 -Δ <sup>6</sup>	18.2	81.8	-	-	-	-
	pAM854 -Δ <sup>6</sup> &Δ <sup>12</sup>	42.7	25.3	19.5	-	16.5	-
18:0, stearic acid; 18:1, oleic acid; 18:2, linoleic acid; 18:3(α), linolenic acid; 18:3(γ), γ-linolenic acid; 18:4, octadecatetraenoic acid							
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**EXAMPLE 4****Transformation of Synechococcus  
with  $\Delta 6$  and  $\Delta 12$  Desaturase Genes**

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A third cosmid, cSy7, which contains a  $\Delta 12$ -desaturase gene, was isolated by screening the Synechocystis genomic library with a oligonucleotide synthesized from the published Synechocystis  $\Delta 12$ -desaturase gene sequence (Wada et al. [1990] Nature (London) 347, 200-203). A 1.7 kb AvaI fragment from this cosmid containing the  $\Delta 12$ -desaturase gene was identified and used as a probe to demonstrate that cSy13 not only contains a  $\Delta 6$ -desaturase gene but also a  $\Delta 12$ -desaturase gene (Figure 3). Genomic Southern blot analysis further showed that both the  $\Delta 6$ -and  $\Delta 12$ -desaturase genes are unique in the Synechocystis genome so that both functional genes involved in C18 fatty acid desaturation are linked closely in the Synechocystis genome.

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The unicellular cyanobacterium Synechococcus (PCC 7942) is deficient in both linoleic acid and GLA(3). The  $\Delta 12$  and  $\Delta 6$ -desaturase genes were cloned individually and together into pAM854 (Bustos et al. [1991] J. Bacteriol. 174, 7525-7533), a shuttle vector that contains sequences necessary for the integration of foreign DNA into the genome of Synechococcus (Golden et al. [1987] Methods in Enzymol. 153, 215-231). Synechococcus was transformed with these gene constructs and colonies were selected. Fatty acid methyl esters were extracted from transgenic Synechococcus and analyzed by GLC.

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1           Table 2 shows that the principal fatty acids  
of wild type Synechococcus are stearic acid (18:0) and  
oleic acid (18:1). Synechococcus transformed with  
pAM854- $\Delta$ 12 expressed linoleic acid (18:2) in addition  
5 to the principal fatty acids. Transformants with  
pAM854- $\Delta$ 6 and  $\Delta$ 12 produced both linoleate and GLA  
(Table 1). These results indicated that Synechococcus  
containing both  $\Delta$ 12- and  $\Delta$ 6-desaturase genes has  
gained the capability of introducing a second double  
10 bond at the  $\Delta$ 12 position and a third double bond at  
the  $\Delta$ 6 position of C18 fatty acids. However, no  
changes in fatty acid composition was observed in the  
transformant containing pAM854- $\Delta$ 6, indicating that in  
the absence of substrate synthesized by the  $\Delta$ 12  
15 desaturase, the  $\Delta$ 6-desaturase is inactive. This  
experiment further confirms that the 1.8 kb  
NheI/HindIII fragment (Figure 3) contains both coding  
and promoter regions of the Synechocystis  $\Delta$ 6-  
desaturase gene. Transgenic Synechococcus with  
20 altered levels of polyunsaturated fatty acids were  
similar to wild type in growth rate and morphology.

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## EXAMPLE 5

Nucleotide Sequence of  $\Delta 6$ -Desaturase

The nucleotide sequence of the 1.8 kb  
5 fragment of cSy75-3.5 including the functional  $\Delta 6$ -  
desaturase gene was determined. An open reading frame  
encoding a polypeptide of 359 amino acids was  
identified (Figure 4). A Kyte-Doolittle hydropathy  
analysis (Kyte et al. [1982] J. Mol. Biol. 157, 105-  
10 132) identified two regions of hydrophobic amino acids  
that could represent transmembrane domains (Figure  
1A); furthermore, the hydropathic profile of the  $\Delta 6$ -  
desaturase is similar to that of the  $\Delta 12$ -desaturase  
gene (Figure 1B; Wada et al.) and  $\Delta 9$ -desaturases  
15 (Thiede et al. [1986] J. Biol. Chem. 261, 13230-  
13235). However, the sequence similarity between the  
Synechocystis  $\Delta 6$ - and  $\Delta 12$ -desaturases is less than 40%  
at the nucleotide level and approximately 18% at the  
amino acid level.

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## EXAMPLE 6

Transfer of Cyanobacterial  $\Delta^6$ -Desaturase into Tobacco

5 The cyanobacterial  $\Delta^6$ -desaturase gene was mobilized into a plant expression vector and transferred to tobacco using Agrobacterium mediated gene transfer techniques. To ensure that the transferred desaturase is appropriately expressed in leaves and developing seeds and that the desaturase

10 gene product is targeted to the endoplasmic reticulum or the chloroplast, various expression cassettes with Synechocystis  $\Delta$ -desaturase open reading frame (ORF) were constructed. Components of these cassettes include: (i) a 35S promoter or seed specific promoter

15 derived from the sunflower helianthinin gene to drive  $\Delta^6$ -desaturase gene expression in all plant tissues or only in developing seeds respectively, (ii) a putative signal peptide either from carrot extensin gene or sunflower helianthinin gene to target newly

20 synthesized  $\Delta^6$ -desaturase into the ER, (iii) an ER lumen retention signal sequence (KDEL) at the COOH-terminal of the  $\Delta^6$ -desaturase ORF, and (iv) an optimized transit peptide to target  $\Delta^6$  desaturase into the chloroplast. The 35S promoter is a derivative of

25 pRTL2 described by Restrepo et al. (1990). The optimized transit peptide sequence is described by Van de Broeck et al. (1985). The carrot extensin signal peptide is described by Chen et al. (1985) EMBO J. 2, 2145.

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Transgenic tobacco plants were produced containing a chimeric cyanobacterial desaturase gene,

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1 comprised of the Synechocystis  $\Delta^6$  desaturase gene fused  
to an endoplasmic reticulum retention sequence (KDEL)  
and extensin signal peptide driven by the CaMV 35S  
promoter. PCR amplifications of transgenic tobacco  
5 genomic DNA indicate that the  $\Delta^6$  desaturase gene was  
incorporated into the tobacco genome. Fatty acid  
methyl esters of leaves of these transgenic tobacco  
plants were extracted and analyzed by Gas Liquid  
Chromatography (GLC). These transgenic tobacco  
10 accumulated significant amounts of GLA (Figure 4).  
Figure 4 shows fatty acid methyl esters as determined  
by GLC. Peaks were identified by comparing the  
elution times with known standards of fatty acid  
methyl ester. Accordingly, cyanobacterial genes  
15 involved in fatty acid metabolism can be used to  
generate transgenic plants with altered fatty acid  
compositions.

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## EXAMPLE 7

## Construction of Borage cDNA library

Membrane bound polysomes were isolated from  
5 borage seeds 12 days post pollination (12 DPP) using  
the protocol established for peas by Larkins and  
Davies (1975 Plant Phys. 55:749-756). RNA was  
extracted from the polysomes as described by Mechler  
(1987 Methods in Enzymology 152:241-248, Academic  
10 Press).

Poly-A+ RNA was isolated from the membrane  
bound polysomal RNA by use of Oligotex-dT beads  
(Qiagen). Corresponding cDNA was made using  
Stratagene's ZAP cDNA synthesis kit. The cDNA library  
15 was constructed in the lambda ZAP II vector  
(Stratagene) using the lambda ZAP II vector kit. The  
primary library was packaged in Gigapack II Gold  
packaging extract (Stratagene). The library was used  
to generate expressed sequence tags (ESTs), and  
20 sequences corresponding to the tags were used to scan  
the GenBank database.

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**EXAMPLE 8**  
**Hybridization Protocol**

Hybridization probes for screening the  
5 borage cDNA library were generated by using random  
primed DNA synthesis as described by Ausubel et al  
(1994 Current Protocols in Molecular Biology, Wiley  
Interscience, N.Y.) and corresponded to previously  
10 identified abundantly expressed seed storage protein  
cDNAs. Unincorporated nucleotides were removed by use  
of a G-50 spin column (Boehringer Mannheim). Probe was  
denatured for hybridization by boiling in a water bath  
for 5 minutes, then quickly cooled on ice. Filters  
for hybridization were prehybridized at 60°C for 2-4  
15 hours in prehybridization solution (6XSSC [Maniatis et  
al 1984 Molecular Cloning A Laboratory Manual, Cold  
Spring Harbor Laboratory], 1X Denharts Solution, 0.05%  
sodium pyrophosphate, 100 µg/ml denatured salmon sperm  
DNA). Denatured probe was added to the hybridization  
20 solution (6X SSC, 1X Denharts solution, 0.05% sodium  
pyrophosphate, 100 µg/ml denatured salmon sperm DNA)  
and incubated at 60°C with agitation overnight.  
Filters were washed in 4x, 2x, and 1x SET washes for  
15 minutes each at 60°C. A 20X SET stock solution is  
25 3M NaCl, 0.4 M Tris base, 20 mM Na<sub>2</sub>EDTA-2H<sub>2</sub>O. The 4X  
SET wash was 4X SET, 12.5 mM PO<sub>4</sub>, pH 6.8 and 0.2% SDS.  
The 2X SET wash was 2X SET, 12.5 mM PO<sub>4</sub>, pH 6.8 and  
0.2% SDS. The 1X SET wash was 1X SET, 12.5 mM PO<sub>4</sub>, pH  
6.8 and 0.2% SDS. Filters were allowed to air dry and  
30 were then exposed to X-ray film for 24 hours with  
intensifying screens at -80°C.

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## EXAMPLE 9

Random sequencing of cDNAs from a borage seed  
(12 DPP) membrane-bound polysomal library

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The borage cDNA library was plated at low density (500 pfu on 150 mm petri dishes). Highly prevalent seed storage protein cDNAs were "subtracted" by screening with the previously identified corresponding cDNAs. Non-hybridizing plaques were excised using Stratagene's excision protocol and reagents. Resulting bacterial colonies were used to inoculate liquid cultures and were either sequenced manually or by an ABI automated sequencer. Each cDNA was sequenced once and a sequence tag generated from 200-300 base pairs. All sequencing was performed by cycle sequencing (Epicentre). Over 300 ESTs were generated. Each sequence tag was compared to GenBank database by BLASTX computer program and a number of lipid metabolism genes, including the  $\Delta 6$ -desaturase were identified.

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Database searches with a cDNA clone designated mbp-65 using BLASTX with the GenBank database resulted in a significant match to the Synechocystis  $\Delta 6$ -desaturase. It was determined however, that this clone was not a full length cDNA. A full length cDNA was isolated using mbp-65 to screen the borage membrane-bound polysomal library. The sequence of the isolated cDNA was determined (Fig. 5A, SEQ ID NO:4) and the protein sequence of the open reading frame (Fig. 5B, SEQ ID NO:5) was compared to other known desaturases using Geneworks

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1 (IntelligGenetics) protein alignment program (Fig. 2).  
This alignment indicated that the cDNA was the borage  
delta-6-desaturase gene.

Although similar to other known plant  
5 desaturases, the borage delta 6-desaturase is distinct  
as indicated in the dendrogram shown in Fig. 6.  
Furthermore, comparison of the amino acid sequences  
characteristic of desaturases, particularly those  
proposed to be involved in metal binding (metal box 1  
10 and metal box 2), illustrates the differences between  
the borage delta 6-desaturase and other plant  
desaturases (Table 3).

The borage delta 6-desaturase is  
distinguished from the cyanobacterial form not only in  
15 over all sequence (Fig. 6) but also in the lipid box,  
metal box 1 and metal box 2 amino acid motifs (Table  
3). As Table 3 indicates, all three motifs are novel  
in sequence. Only the borage delta 6-desaturase metal  
box 2 shown some relationship to the Synechocystis  
20 delta-6 desaturase metal box 2.

In addition, the borage delta 6-desaturase  
is also distinct from another borage desaturase gene,  
the delta-12 desaturase. P1-81 is a full length cDNA  
that was identified by EST analysis and shows high  
25 similarity to the Arabidopsis delta-12 desaturase (Fad  
2). A comparison of the lipid box, metal box 1 and  
metal box 2 amino acid motifs (Table 3) in borage  
delta 6 and delta-12 desaturases indicates that little  
homology exists in these regions. The placement of  
30 the two sequences in the dendrogram in Fig. 6  
indicates how distantly related these two genes are.

Table 3. Comparison of common amino acid motifs in membrane-bound desaturases

Desaturase	Amino Acid Motif				Metal Box 1		Metal Box 2	
	Lipid Box							
Borage $\Delta^6$	WIGHDAGH (SEQ. ID. NO: 6)	HNAHH (SEQ. ID. NO: 12)	FOIEHH (SEQ. ID. NO: 20)					
Synechocystis $\Delta^6$	NVGHDANH (SEQ. ID. NO: 7)	HNYLHH (SEQ. ID. NO: 13)	HQVTHH (SEQ. ID. NO: 21)					
Arab. chloroplast $\Delta^{11}$	VLGHDCGH (SEQ. ID. NO: 8)	HRTHH (SEQ. ID. NO: 14)	HVHHH (SEQ. ID. NO: 22)					
Rice $\Delta^{15}$	VLGHDCGH (SEQ. ID. NO: 8)	HRTHH (SEQ. ID. NO: 14)	HVHHH (SEQ. ID. NO: 22)					
Glycine chloroplast $\Delta^{15}$	VLGHDCGH (SEQ. ID. NO: 8)	HRTHH (SEQ. ID. NO: 14)	HVHHH (SEQ. ID. NO: 22)					
Arab. fad3 ( $\Delta^{15}$ )	VLGHDCGH (SEQ. ID. NO: 8)	HRTHH (SEQ. ID. NO: 14)	HVHHH (SEQ. ID. NO: 22)					
Brassica fad3 ( $\Delta^{15}$ )	VLGHDCGH (SEQ. ID. NO: 8)	HRTHH (SEQ. ID. NO: 14)	HVHHH (SEQ. ID. NO: 22)					
Borage $\Delta^{12}$ (P1-81)*	VIAHECGH (SEQ. ID. NO: 9)	HRRHH (SEQ. ID. NO: 15)	HVAHH (SEQ. ID. NO: 23)					
Arab. fad2 ( $\Delta^{17}$ )	VIAHECGH (SEQ. ID. NO: 9)	HRRHH (SEQ. ID. NO: 15)	HVAHH (SEQ. ID. NO: 23)					
Arab. chloroplast $\Delta^{12}$	VIGHDCAH (SEQ. ID. NO: 10)	HDRHH (SEQ. ID. NO: 16)	HIPHH (SEQ. ID. NO: 24)					
Glycine plastid $\Delta^{12}$	VIGHDCAH (SEQ. ID. NO: 10)	HDRHH (SEQ. ID. NO: 16)	HIPHH (SEQ. ID. NO: 24)					
Spinach plastidial n-6	VIGHDCAH (SEQ. ID. NO: 10)	HDQHH (SEQ. ID. NO: 17)	HIPHH (SEQ. ID. NO: 24)					
Synechocystis $\Delta^{12}$	VVGHDGCH (SEQ. ID. NO: 11)	HDWHH (SEQ. ID. NO: 18)	HIPHH (SEQ. ID. NO: 24)					
Anabaena $\Delta^{12}$	VLGHDCGH (SEQ. ID. NO: 8)	HNHHH (SEQ. ID. NO: 19)	HVPHH (SEQ. ID. NO: 25)					

\*P1-81 is a full length cDNA which was identified by EST analysis and shows high similarity to the Arabidopsis  $\Delta^{12}$  desaturase (fad2)

## EXAMPLE 10

1 Construction of 222.1Δ<sup>6</sup>NOS for transient  
and expression

5 The vector pBI221 (Jefferson et al. 1987  
EMBO J. 6:3901-3907) was prepared for ligation by  
digestion with BamHI and EcoICR I (Promega) which  
excises the GUS coding region leaving the 35S promoter  
and NOS terminator intact. The borage Δ<sup>6</sup>-desaturase  
10 cDNA was excised from the Bluescript plasmid  
(Stratagene) by digestion with BamHI and XhoI. The  
XhoI end was made blunt by use of the Klenow fragment.  
This fragment was then cloned into the BamHI/EcoICR I  
sites of pBI221, yielding 221.Δ<sup>6</sup>NOS (Fig. 7). In  
221.Δ<sup>6</sup>.NOS, the remaining portion (backbone) of the  
15 restriction map depicted in Fig. 7 is pBI221.

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**EXAMPLE 11****Construction of 121. $\Delta^6$ .NOS for stable transformation**

The vector pBI121 (Jefferson et al. 1987  
5 EMBO J. 6:3901-3907) was prepared for ligation by  
digestion with BamHI and EcoICR I (Promega) which  
excises the GUS coding region leaving the 35S promoter  
and NOS terminator intact. The borage  $\Delta^6$ -desaturase  
cDNA was excised from the Bluescript plasmid  
10 (Stratagene) by digestion with BamHI and XhoI. The  
XhoI end was made blunt by use of the Klenow fragment.  
This fragment was then cloned into the BamHI/EcoICR I  
sites of pBI121, yielding 121.1 $\Delta^6$ NOS (Fig. 7). In  
121. $\Delta^6$ .NOS, the remaining portion (backbone) of the  
15 restriction map depicted in Fig. 7 is pBI121.

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**EXAMPLE 12****Transient Expression**

5 All work involving protoplasts was performed  
in a sterile hood. One ml of packed carrot suspension  
cells were digested in 30 mls plasmolyzing solution  
(25 g/l KCl, 3.5 g/l  $\text{CaCl}_2 \cdot \text{H}_2\text{O}$ , 10mM MES, pH 5.6 and  
0.2 M mannitol) with 1% cellulase, 0.1% pectolyase,  
10 and 0.1% dreisalase overnight, in the dark, at room  
temperature. Released protoplasts were filtered  
through a 150  $\mu\text{m}$  mesh and pelleted by centrifugation  
(100x g, 5 min.) then washed twice in plasmolyzing  
solution. Protoplasts were counted using a double  
15 chambered hemocytometer. DNA was transfected into the  
protoplasts by PEG treatment as described by Nunberg  
and Thomas (1993 Methods in Plant Molecular Biology  
and Biotechnology, B.R. Glick and J.E. Thompson, eds.  
pp. 241-248) using  $10^6$  protoplasts and 50-70 ug of  
20 plasmid DNA (221.Δ6.NOS). Protoplasts were cultured  
in 5 mls of MS media supplemented with 0.2M mannitol  
and 3  $\mu\text{M}$  2,4-D for 48 hours in the dark with shaking.

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## EXAMPLE 13

## Stable transformation of tobacco

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121.Δ<sup>6</sup>.NOS plasmid construction was used to transform tobacco (*Nicotiana tabacum* cv. xanthi) via Agrobacterium according to standard procedures (Horsh et al., 1985 Science 227: 1229-1231; Bogue et al., 1990 Mol. Gen. Genet. 221:49-57), except that initial transformants were selected on 100 ug/ml kanamycin.

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**EXAMPLE 14****Preparation and analysis of  
fatty acid methyl esters (FAMES)**

5 Tissue from transfected protoplasts and  
transformed tobacco plants was frozen in liquid  
nitrogen and lyophilized overnight. FAMES were  
prepared as described by Dahmer et al (1989 J. Amer.  
Oil Chem. Soc. 66:543-548). In some cases, the  
solvent was evaporated again, and the FAMES were  
10 resuspended in ethyl acetate and extracted once with  
deionized water to remove any water soluble  
contaminants. The FAMES were analyzed by gas  
chromatography (GC) on a J&W Scientific DB-wax column  
(30 m length, 0.25 mm ID, 0.25  $\mu$ m film).

15 An example of a transient assay is shown in  
Fig. 8 which represents three independent  
transfections pooled together. The addition of the  
borage  $\Delta 6$ -desaturase cDNA corresponds with the  
appearance of gamma linolenic acid (GLA) which is one  
20 of the possible products of  $\Delta 6$ -desaturase.

Figures 9 and 10 depict GC profiles of the  
FAMES derived from leaf and seed tissue, respectively,  
of control and transformed tobacco plants. Figure 9A  
provides the profile of leaf tissue of wild-type  
25 tobacco (xanthi); Figure 9B provides the profile of  
leaf tissue from a tobacco plant transformed with the  
borage  $\Delta$ -6 desaturase under the transcriptional  
control of the 35S CaMV promoter (pBI 121 $\Delta$ <sup>6</sup>NOS).  
Peaks correspond to 18:2, 18:3 $\gamma$  (GLA), 18:3 $\alpha$  and 18:4  
30 (octadecanonic acid). Figure 10A shows the GC profile  
of seeds of a wild-type tobacco; Figure 10B shows the

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- 1 profile of seed tissue of a tobacco plant transformed  
with pBI 121 $\Delta^6$ NOS. Peaks correspond to 18:2,  
18:3 $\gamma$ (GLA) and 18:3 $\alpha$ .

- 5 The relative distribution of the C<sub>18</sub> fatty  
acids in control and transgenic tobacco seeds is shown  
in Table 4.

TABLE 4

	Fatty Acid	Xanthi	pBI121 $\Delta^6$ NOS
10	18:0	4.0%	2.5%
	18:1	13%	13%
	18:2	82%	82%
	18:3 $\gamma$ (GLA)	-	2.7%
15	18:3 $\alpha$	0.82%	1.4%

- 20 The foregoing results demonstrate that GLA  
is incorporated into the triacylglycerides of  
transgenic tobacco leaves and seeds containing the  
borage  $\Delta^6$ -desaturase.

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## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

- (i) APPLICANT: Rhone-Poulenc Agrochimie
- (ii) TITLE OF INVENTION: PRODUCTION OF GAMMA LINOLENIC ACID BY A  
DELTA 6-DESATURASE
- (iii) NUMBER OF SEQUENCES: 25
- (iv) CORRESPONDENCE ADDRESS:
  - (A) ADDRESSEE: Scully, Scott, Murphy & Presser
  - (B) STREET: 400 Garden City Plaza
  - (C) CITY: Garden City
  - (D) STATE: New York
  - (E) COUNTRY: United States
  - (F) ZIP: 11530
- (v) COMPUTER READABLE FORM:
  - (A) MEDIUM TYPE: Floppy disk
  - (B) COMPUTER: IBM PC compatible
  - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
  - (D) SOFTWARE: PatentIn Release #1.0, Version #1.25
- (vi) CURRENT APPLICATION DATA:
  - (A) APPLICATION NUMBER:
  - (B) FILING DATE: 30-DEC-1994
  - (C) CLASSIFICATION:
- (viii) ATTORNEY/AGENT INFORMATION:
  - (A) NAME: Presser, Leopold
  - (B) REGISTRATION NUMBER: 19,827
  - (C) REFERENCE/DOCKET NUMBER: 8383ZYXW
- (ix) TELECOMMUNICATION INFORMATION:
  - (A) TELEPHONE: (516) 742-4343
  - (B) TELEFAX: (516) 742-4366
  - (C) TELEX: 230 901 SANS UR

## (2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 3588 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: both
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)

## (ix) FEATURE:

(A) NAME/KEY: CDS  
(B) LOCATION: 2002..3081

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GCTAGCCACC AGTGACGATG CCTTGAATTT GGCCATTCTG ACCCAGGCCC GTATTCTGAA	60
TCCCCGCATT CGCATTGTTA ATCGTTTGTG CAACCATGCC CTGGGTAAAC GTTTAGACAC	120
CACCTTGCCA GACCACGTTA GTTTGAGTGT TTCCGCCCTG GCGGCCCCGA TTTTTCCTT	180
TGCGGCTTTG GGCAATCAGG CGATCGGGCA ATTGCGTTTG TTTGACCAGA CTTGGCCCAT	240
TCAGGAAATT GTCATTCACC AAGACCATCC CTGGCTCAAT TTACCCCTGG CGGATTTATG	300
GGATGATCCG AGCCGAATGT TGATCTATTA CCTACCGGCC CACAGTGAAA CGGATTTAGT	360
AGGCGCAGTG GTGAATAATT TAACGTTGCA ATCTGGGGAC CATTTAATAG TGGGACAAAA	420
ACCCCAACCC AAGACCAAAC GGCGATCGCC TTGGCGCAA TTTTCCAAAC TGATTACCAA	480
CCTGCGGGAG TATCAGCGGT ATGTCCAACA GGTGATATGG GTGGTGTGTG TTTTATTGTT	540
GATGATTTTT CTGGCCACCT TCATCTACGT TTCCATTGAT CAACATATTG CCCCAGTGGA	600
CGCGTTGTAT TTTTCCGTGG GCATGATTAC CGGGGCCGGT GGCAAGGAAG AGGTGGCCGA	660
AAAGTCCCCC GATATCATCA AAGTATTCAC AGTGGTGATG ATGATCGCCG GGGCGGGGGT	720
GATTGGTATT TGTTATGCCC TACTGAATGA TTTCATCCTT GGCAGTCGCT TTAGTCAGTT	780
TTTGGATGCG GCCAAGTTAC CCGATCGCCA TCACATCATC ATTTGTGGGC TGGGGGGAGT	840
GAGCATGGCC ATTATTGAAG AGTTAATTCA CCAGGGCCAT GAAATTGTGG TAATCGAAAA	900
GGATACAGAT AATCGTTTCT TGCATACGGC CCGCTCCCTG GGGGTGCCCG TAATTGTGGA	960
GGATGCCCCG CTAGAAAGAA CGTTGGCCTG CGCCAATATC AACCAGAGCCG AAGCCATTGT	1020
GGTGGCCACC AGCGACGACA CCGTTAACTT GGAAATTGGC CTAAGTGCCA AGGCGATCGC	1080
CCCTAGCCTG CCAGTGGTGT TCGGTTGCCA GGATGCCCAG TTTAGCCTGT CCCTGCAGGA	1140
AGTATTTGAA TTTGAAACGG TGCTTTGTCC GGCAGGAATTG GCCACCTATT CCTTTGCGGC	1200
GGCGGCCCTG GGGGGCAAAA TTTGGGGCAA CGGCATGACC GATGATTGTC TGTGGGTAGC	1260
CCTAGCCACC TTAATCACTC CTAACCATCC CTTTGCCGAC CAATTGGTTA AAATTGCAGC	1320
CCAAAAGTCT GATTTTCGTC CCCTCTATCT AGAACGGGGT GGCAAAACCA TCCATAGCTG	1380
GGAATTATTG GGTACCCATC TCGACTCTGG AGACGTGTTG TATTTAACCA TGCCCCGCCAC	1440
TGCCCTAGAG CAACTTTGGC GATCGCCCCG TGCCACTGCT GATCCTCTGG ACTCTTTTTT	1500

GGTTTAGCAT GGGGGGATGG AACTCTTGAC TCGGCCCAAT GGTGATCAAG AAAGAACGCT	1560
TTGTCTATGT TTAGTATTTT TAAGTTAACC AACAGCAGAG GATAACTTCC AAAAGAAAATT	1620
AAGCTCAAAA AGTAGCAAAA TAAGTTTAAT TCATAACTGA GTTTTACTGC TAAACAGCGG	1680
TGCAAAAAAAG TCAGATAAAA TAAAAGCTTC ACTTCGGTTT TATATTGTGA CCATGGTTCC	1740
CAGGCATCTG CTCTAGGGAG TTTTCCGCT GCCTTTAGAG AGTATTTTCT CCAAGTCGGC	1800
TAACTCCCCC ATTTTTAGGC AAAATCATAT ACAGACTATC CCAATATTGC CAGAGCTTTG	1860
ATGACTCACT GTAGAAGGCA GACTAAAATT CTAGCAATGG ACTCCCAGTT GGAATAAATT	1920
TTTAGTCTCC CCCGGCGCTG GAGTTTTTTT GTAGTTAATG GCGGTATAAT GTGAAAGTTT	1980
TTTATCTATT TAAATTTATA A ATG CTA ACA GCG GAA AGA ATT AAA TTT ACC	2031
Met Leu Thr Ala Glu Arg Ile Lys Phe Thr	
1 5 10	
CAG AAA CGG GGG TTT CGT CGG GTA CTA AAC CAA CGG GTG GAT GCC TAC	2079
Gln Lys Arg Gly Phe Arg Arg Val Leu Asn Gln Arg Val Asp Ala Tyr	
15 20 25	
TTT GCC GAG CAT GGC CTG ACC CAA AGG GAT AAT CCC TCC ATG TAT CTG	2127
Phe Ala Glu His Gly Leu Thr Gln Arg Asp Asn Pro Ser Met Tyr Leu	
30 35 40	
AAA ACC CTG ATT ATT GTG CTC TGG TTG TTT TCC GCT TGG GCC TTT GTG	2175
Lys Thr Leu Ile Ile Val Leu Trp Leu Phe Ser Ala Trp Ala Phe Val	
45 50 55	
CTT TTT GCT CCA GTT ATT TTT CCG GTG CGC CTA CTG GGT TGT ATG GTT	2223
Leu Phe Ala Pro Val Ile Phe Pro Val Arg Leu Gly Cys Met Val	
60 65 70	
TTG GCG ATC GCC TTG GCG GCC TTT TCC TTC AAT GTC GGC CAC GAT GCC	2271
Leu Ala Ile Ala Leu Ala Ala Phe Ser Phe Asn Val Gly His Asp Ala	
75 80 85 90	
AAC CAC AAT GCC TAT TCC TCC AAT CCC CAC ATC AAC CGG GTT CTG GGC	2319
Asn His Asn Ala Tyr Ser Ser Asn Pro His Ile Asn Arg Val Leu Gly	
95 100 105	
ATG ACC TAC GAT TTT GTC GGG TTA TCT AGT TTT CTT TGG CGC TAT CGC	2367
Met Thr Tyr Asp Phe Val Gly Leu Ser Ser Phe Leu Trp Arg Tyr Arg	
110 115 120	
CAC AAC TAT TTG CAC CAC ACC TAC ACC AAT ATT CTT GGC CAT GAC GTG	2415
His Asn Tyr Leu His His Thr Tyr Thr Asn Ile Leu Gly His Asp Val	
125 130 135	
GAA ATC CAT GGA GAT GGC GCA GTA CGT ATG AGT CCT GAA CAA GAA CAT	2463
Glu Ile His Gly Asp Gly Ala Val Arg Met Ser Pro Glu Gln Glu His	
140 145 150	





TTTGAGGGGG TTCATTGGCC GCAGTTTCAA GCTGACCTAG GAGGCAAAGA TTGGGTGATT 3268  
 TTGCTCAAAT CCGCTGGGAT ATTGAAAGGC TTCACCACCT TTGGTTTCTA CCCTGCTCAA 3328  
 TGGGAAGGAC AAACCGTCAG AATTGTTTAT TCTGGTGACA CCATCACCGA CCCATCCATG 3388  
 TGGTCTAACC CAGCCCTGGC CAAGGCTTGG ACCAAGGCCA TGCAAATTCT CCACGAGGCT 3448  
 AGGCCAGAAA AATTATATTG GCTCCTGATT TCTCCGGCT ATCGCACCTA CCGATTTTGT 3508  
 AGCATTTTGT CCAAGGAATT CTATCCCCAC TATCTCCATC CCACTCCCCC GCCTGTACAA 3568  
 AATTTTATCC ATCAGCTAGC 3588

## (2) INFORMATION FOR SEQ ID NO:2:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 359 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Leu Thr Ala Glu Arg Ile Lys Phe Thr Gln Lys Arg Gly Phe Arg  
 1 5 10 15  
 Arg Val Leu Asn Gln Arg Val Asp Ala Tyr Phe Ala Glu His Gly Leu  
 20 25 30  
 Thr Gln Arg Asp Asn Pro Ser Met Tyr Leu Lys Thr Leu Ile Ile Val  
 35 40 45  
 Leu Trp Leu Phe Ser Ala Trp Ala Phe Val Leu Phe Ala Pro Val Ile  
 50 55 60  
 Phe Pro Val Arg Leu Leu Gly Cys Met Val Leu Ala Ile Ala Leu Ala  
 65 70 75 80  
 Ala Phe Ser Phe Asn Val Gly His Asp Ala Asn His Asn Ala Tyr Ser  
 85 90 95  
 Ser Asn Pro His Ile Asn Arg Val Leu Gly Met Thr Tyr Asp Phe Val  
 100 105 110  
 Gly Leu Ser Ser Phe Leu Trp Arg Tyr Arg His Asn Tyr Leu His His  
 115 120 125  
 Thr Tyr Thr Asn Ile Leu Gly His Asp Val Glu Ile His Gly Asp Gly  
 130 135 140  
 Ala Val Arg Met Ser Pro Glu Gln Glu His Val Gly Ile Tyr Arg Phe  
 145 150 155 160

**(i) SEQUENCE CHARACTERISTICS:**

- (A) LENGTH: 1884 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: both  
(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

AGCTTCACTT CGGTTTTATA TTGTGACCAT GGTTCCCAGG CATCTGCTCT AGGGAGTTTT 60  
TCCGCTGCCT TTAGAGAGTA TTTTCTCCAA GTCGGCTAAC TCCCCATTT TTAGGCAAAA 120

TCATATACAG ACTATCCCAA TATTGCCAGA GCTTTGATGA CTCACTGTAG AAGGCAGACT 180  
AAAATTCTAG CAATGGACTC CCAGTTGGAA TAAATTTTTTA GTCTCCCCCG GCGCTGGAGT 240  
TTTTTTGTAG TTAATGGCGG TATAATGTGA AAGTTTTTTA TCTATTTAAA TTTATAAATG 300  
CTAACAGCGG AAAGAATTAA ATTTACCCAG AAACGGGGGT TTCGTCGGGT ACTAAACCAA 360  
CGGGTGGATG CCTACTTTGC CGAGCATGGC CTGACCCAAA GGGATAATCC CTCCATGTAT 420  
CTGAAAACCC TGATTATTGT GCTCTGGTTG TTTTCCGCTT GGGCCTTTGT GCTTTTTGCT 480  
CCAGTTATTT TTCCGGTGCG CCTACTGGGT TGTATGGTTT TGGCGATCGC CTGGCGGGCC 540  
TTTTCCCTTCA ATGTCGGCCA CGATGCCAAC CACAATGCCT ATTCTCCAA TCCCCACATC 600  
AACCGGGTTC TGGGCATGAC CTACGATTTT GTCGGGTTAT CTAGTTTTCT TTGGCGCTAT 660  
CGCCACAACT ATTTGCACCA CACCTACACC AATATTCTTG GCCATGACGT GGAAATCCAT 720  
GGAGATGGCG CAGTACGTAT GAGTCCTGAA CAAGAACATG TTGGTATTTA TCGTTTCCAG 780  
CAATTTTATA TTTGGGGTTT ATATCTTTTC ATTCCCTTTT ATTGGTTTCT CTACGATGTC 840  
TACCTAGTGC TTAATAAAGG CAAATATCAC GACCATAAAA TTCCTCCTTT CCAGCCCCTA 900  
GAATTAGCTA GTTTGCTAGG GATTAAGCTA TTATGGCTCG GCTACGTTTT CGGCTTACCT 960  
CTGGCTCTGG GCTTTTCCAT TCCTGAAGTA TTAATTGGTG CTTCCGGTAAC CTATATGACC 1020  
TATGGCATCG TGGTTTGAC CATCTTTATG CTGGCCCATG TGTTGGAATC AACTGAATTT 1080  
CTCACCCCCG ATGGTGAATC CGGTGCCATT GATGACGAGT GGGCTATTTG CCAAATTCGT 1140  
ACCACGGCCA ATTTTGCCAC CAATAATCCC TTTTGGAAT GGTTTTGTGG CGGTTTAAAT 1200  
CACCAAGTTA CCCACCATCT TTTCCCCAAT ATTTGTCATA TTCCTATCC CCAATTGGAA 1260  
AATATTATTA AGGATGTTTG CCAAGAGTTT GGTGTGGAAT ATAAAGTTTA TCCCACCTTC 1320  
AAAGCGGCGA TCGCCTCTAA CTATCGCTGG CTAGAGGCCA TGGGCAAAGC ATCGTGACAT 1380  
TGCCCTGGGA TTGAAGCAAA ATGGCAAAAT CCCTCGTAAA TCTATGATCG AAGCCTTTCT 1440  
GTTGCCCCGCC GACCAAATCC CCGATGCTGA CCAAAGGTTG ATGTTGGCAT TGCTCCAAAC 1500  
CCACTTTGAG GGGGTTTATT GGCCGCAGTT TCAAGCTGAC CTAGGAGGCA AAGATTGGGT 1560  
GATTTTGCTC AAATCCGCTG GGATATTGAA AGGCTTCACC ACCTTTGGTT TCTACCCTGC 1620  
TCAATGGGAA GGACAAACCG TCAGAATTGT TTATTCTGGT GACACCATCA CCGACCCATC 1680  
CATGTGGTCT AACCCAGCCC TGGCCAAGGC TTGGACCAAG GCCATGCAAA TTCTCCACGA 1740  
GGCTAGGCCA GAAAAATTAT ATTGGCTCCT GATTTCTTCC GGCTATCGCA CCTACCGATT 1800

TTTGAGCATT TTTGCCAAGG AATTCTATCC CCACTATCTC CATCCCACTC CCCC GCCTGT 1860  
 AAAAAATTTT ATCCATCAGC TAGC 1884

## (2) INFORMATION FOR SEQ ID NO:4:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1685 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: both
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

AATATCTGCC TACCCTCCCA AAGAGAGTAG TCATTTTTTCA TCAATGGCTG CTCAAATCAA	60
GAAATACATT ACCTCAGATG AACTCAAGAA CCACGATAAA CCCGGAGATC TATGGATCTC	120
GATTCAAGGG AAAGCCTATG ATGTTTCGGA TTGGGTGAAA GACCATCCAG GTGGCAGCTT	180
TCCCTTGAAG AGTCTTGCTG GTCAAGAGGT AACTGATGCA TTTGTTGCAT TCCATCCTGC	240
CTCTACATGG AAGAATCTTG ATAAGTTTTT CACTGGGTAT TATCTTAAAG ATTACTCTGT	300
TTCTGAGGTT TCTAAAGATT ATAGGAAGCT TGTGTTTGAG TTTTCTAAAA TGGGTTTGTA	360
TGACAAAAAA GGTCAATATTA TGTTTGCAAC TTTGTGCTTT ATAGCAATGC TGTTTGCTAT	420
GAGTGTTTAT GGGGTTTTGT TTTGTGAGGG TGTTTGGTA CATTTGTTTT CTGGGTGTTT	480
GATGGGGTTT CTTTGGATTC AGAGTGGTTG GATTGGACAT GATGCTGGGC ATTATATGGT	540
AGTGTCTGAT TCAAGGCTTA ATAAGTTTAT GGGTATTTTT GCTGCAAATT GTCTTTCAGG	600
AATAAGTATT GGTGGTGGA AATGGAACCA TAATGCACAT CACATTGCCT GTAATAGCCT	660
TGAATATGAC CCTGATTTAC AATATATACC ATTCCTTGTT GTGTCTTCCA AGTTTTTTGG	720
TTCACTCACC TCTCATTTCT ATGAGAAAAG GTTGACTTTT GACTCTTTAT CAAGATTCTT	780
TGTAAGTTAT CAACATTGGA CATTTTACCC TATTATGTGT GCTGCTAGGC TCAATATGTA	840
TGTACAATCT CTCATAATGT TGTGACCAA GAGAAATGTG TCCTATCGAG CTCAGGAACT	900
CTTGGGATGC CTAGTGTTCT CGATTGGTA CCCGTTGCTT GTTTCTTGTT TGCCTAATTG	960
GGGTGAAAGA ATTATGTTTG TTATTGCAAG TTTATCAGTG ACTGGAATGC AACAAGTTCA	1020
GTTCTCCTTG AACCATTCTT CTTCAAGTGT TTATGTTGGA AAGCCTAAAG GGAATAATTG	1080
GTTTGAGAAA CAAACGGATG GGACACTTGA CATTTCTTGT CCTCCTTGGA TGGATTGGTT	1140
TCATGGTGGA TTGCAATTCC AAATTGAGCA TCATTTGTTT CCAAGATGC CTAGATGCAA	1200

CCTTAGGAAA ATCTCGCCCT ACGTGATCGA GTTATGCAAG AAACATAATT TGCCTTACAA 1260  
 TTATGCATCT TTCTCCAAGG CCAATGAAAT GACACTCAGA ACATTGAGGA ACACAGCATT 1320  
 GCAGGCTAGG GATATAACCA AGCCGCTCCC GAAGAATTTG GTATGGGAAG CTCTTCACAC 1380  
 TCATGGTTAA AATTACCCCT AGTTCATGTA ATAATTTGAG ATTATGTATC TCCTATGTTT 1440  
 GTGTCTTGTC TTGGTTCTAC TTGTTGGAGT CATTGCAACT TGTCTTTTAT GGTTTATTAG 1500  
 ATGTTTTTTA ATATATTTTA GAGGTTTTGC TTTCATCTCC ATTATTGATG AATAAGGAGT 1560  
 TGCATATTGT CAATTGTTGT GCTCAATATC TGATATTTTG GAATGTACTT TGTACCACTG 1620  
 TGTTTTCAGT TGAAGCTCAT GTGTACTTCT ATAGACTTTG TTAAATGGT TATGTCATGT 1680  
 TATTT 1685

## (2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 448 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Met Ala Ala Gln Ile Lys Lys Tyr Ile Thr Ser Asp Glu Leu Lys Asn  
 1 5 10 15  
 His Asp Lys Pro Gly Asp Leu Trp Ile Ser Ile Gln Gly Lys Ala Tyr  
 20 25 30  
 Asp Val Ser Asp Trp Val Lys Asp His Pro Gly Gly Ser Phe Pro Leu  
 35 40 45  
 Lys Ser Leu Ala Gly Gln Glu Val Thr Asp Ala Phe Val Ala Phe His  
 50 55 60  
 Pro Ala Ser Thr Trp Lys Asn Leu Asp Lys Phe Phe Thr Gly Tyr Tyr  
 65 70 75 80  
 Leu Lys Asp Tyr Ser Val Ser Glu Val Ser Lys Asp Tyr Arg Lys Leu  
 85 90 95  
 Val Phe Glu Phe Ser Lys Met Gly Leu Tyr Asp Lys Lys Gly His Ile  
 100 105 110  
 Met Phe Ala Thr Leu Cys Phe Ile Ala Met Leu Phe Ala Met Ser Val  
 115 120 125  
 Tyr Gly Val Leu Phe Cys Glu Gly Val Leu Val His Leu Phe Ser Gly  
 130 135 140

- 53 -

Cys Leu Met Gly Phe Leu Trp Ile Gln Ser Gly Trp Ile Gly His Asp  
 145 150 155 160  
 Ala Gly His Tyr Met Val Val Ser Asp Ser Arg Leu Asn Lys Phe Met  
 165 170 175  
 Gly Ile Phe Ala Ala Asn Cys Leu Ser Gly Ile Ser Ile Gly Trp Trp  
 180 185 190  
 Lys Trp Asn His Asn Ala His His Ile Ala Cys Asn Ser Leu Glu Tyr  
 195 200 205  
 Asp Pro Asp Leu Gln Tyr Ile Pro Phe Leu Val Val Ser Ser Lys Phe  
 210 215 220  
 Phe Gly Ser Leu Thr Ser His Phe Tyr Glu Lys Arg Leu Thr Phe Asp  
 225 230 235 240  
 Ser Leu Ser Arg Phe Phe Val Ser Tyr Gln His Trp Thr Phe Tyr Pro  
 245 250 255  
 Ile Met Cys Ala Ala Arg Leu Asn Met Tyr Val Gln Ser Leu Ile Met  
 260 265 270  
 Leu Leu Thr Lys Arg Asn Val Ser Tyr Arg Ala Gln Glu Leu Leu Gly  
 275 280 285  
 Cys Leu Val Phe Ser Ile Trp Tyr Pro Leu Leu Val Ser Cys Leu Pro  
 290 295 300  
 Asn Trp Gly Glu Arg Ile Met Phe Val Ile Ala Ser Leu Ser Val Thr  
 305 310 315 320  
 Gly Met Gln Gln Val Gln Phe Ser Leu Asn His Phe Ser Ser Ser Val  
 325 330 335  
 Tyr Val Gly Lys Pro Lys Gly Asn Asn Trp Phe Glu Lys Gln Thr Asp  
 340 345 350  
 Gly Thr Leu Asp Ile Ser Cys Pro Pro Trp Met Asp Trp Phe His Gly  
 355 360 365  
 Gly Ser Gln Phe Gln Ile Glu His His Leu Phe Pro Lys Met Pro Arg  
 370 375 380  
 Cys Asn Leu Arg Lys Ile Ser Pro Tyr Val Ile Glu Leu Cys Lys Lys  
 385 390 395 400  
 His Asn Leu Pro Tyr Asn Tyr Ala Ser Phe Ser Lys Ala Asn Glu Met  
 405 410 415  
 Thr Leu Arg Thr Leu Arg Asn Thr Ala Leu Gln Ala Arg Asp Ile Thr  
 420 425 430  
 Lys Pro Leu Pro Lys Asn Leu Val Trp Glu Ala Leu His Thr His Gly  
 435 440 445

## (2) INFORMATION FOR SEQ ID NO:6:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 8 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Trp Ile Gly His Asp Ala Gly His  
1 5

## (2) INFORMATION FOR SEQ ID NO:7:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 8 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Asn Val Gly His Asp Ala Asn His  
1 5

## (2) INFORMATION FOR SEQ ID NO:8:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 8 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Val Leu Gly His Asp Cys Gly His  
1 5

## (2) INFORMATION FOR SEQ ID NO:9:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 8 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:



Val Ile Ala His Glu Cys Gly His  
1 5

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 8 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Val Ile Gly His Asp Cys Ala His  
1 5

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 8 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Val Val Gly His Asp Cys Gly His  
1 5

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 5 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

His Asn Ala His His  
1 5

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

His Asn Tyr Leu His His  
1 5

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 5 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

His Arg Thr His His  
1 5

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 5 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

His Arg Arg His His  
1 5

## (2) INFORMATION FOR SEQ ID NO:16:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 5 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

His Asp Arg His His  
1 5

## (2) INFORMATION FOR SEQ ID NO:17:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 5 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

His Asp Gln His His  
1 5

## (2) INFORMATION FOR SEQ ID NO:18:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 5 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

His Asp His His His  
1 5

## (2) INFORMATION FOR SEQ ID NO:19:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 5 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

His Asn His His His  
1 5

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 6 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Phe Gln Ile Glu His His  
1 5

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 6 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

His Gln Val Thr His His  
1 5

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 5 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

His Val Ile His His  
1 5

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 5 amino acids  
(B) TYPE: amino acid

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(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

His Val Ala His His  
1 5

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 5 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

His Ile Pro His His  
1 5

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 5 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

His Val Pro His His  
1 5

1 WHAT IS CLAIMED:

1. An isolated nucleic acid encoding a borage  $\Delta 6$ -desaturase.

5

2. The isolated nucleic acid of Claim 1 comprising the nucleotide sequence of SEQ ID NO: 4.

3. An isolated nucleic acid that codes for the amino acid sequence of SEQ ID NO: 5.

10

4. A vector comprising the nucleic acid of any one Claims 1-3.

15

5. An expression vector comprising the isolated nucleic acid of any one of Claims 1-3 operably linked to a promoter and optionally a termination signal capable of effecting expression of the gene product of said isolated nucleic acid.

20

6. The expression vector of Claim 5 wherein said promoter is a  $\Delta$ -6 desaturase promoter, an Anabaena carboxylase promoter, a helianthinin promoter, a glycinin promoter, a napin promoter, the 35S promoter from CaMV, or a helianthinin tissue-specific promoter.

25

7. The expression vector of Claim 5 wherein said promoter is constitutive or tissue-specific.

30

8. The expression vector of Claim 5 wherein said termination signal is a Synechocystis termination

35

1 signal, a nopaline synthase termination signal, or a seed  
termination signal.

5 9. A cell comprising the vector of any one of  
Claims 4-8.

10 10. The cell of Claim 9 wherein said cell is an  
animal cell, a bacterial cell, a plant cell or a fungal  
cell.

11. A transgenic organism comprising the  
isolated nucleic acid of any one of Claims 1-3.

15 12. A transgenic organism comprising the vector  
of any one of Claims 4-8.

20 13. The transgenic organism of Claim 11 or 12  
wherein said organism is a bacterium, a fungus, a plant or  
an animal.

14. A plant or progeny of said plant which has  
been regenerated from the plant cell of Claim 10.

25 15. The plant of Claim 14 wherein said plant is  
a sunflower, soybean, maize, tobacco, peanut, carrot or  
oil seed rape plant.

30 16. A method of producing a plant with  
increased gamma linolenic acid (GLA) content which  
comprises:

- 1 (a) transforming a plant cell with the isolated  
nucleic acid of any one of Claims 1-3; and  
(b) regenerating a plant with increased GLA  
content from said plant cell.

5

17. A method of producing a plant with  
increased gamma linolenic acid (GLA) content which  
comprises:

- 10 (a) transforming a plant cell with the vector of  
any one of Claims 4-8; and  
(b) regenerating a plant with increased GLA  
content from said plant cell.

18. The method of Claim 16 or 17 wherein said  
15 plant is a sunflower, soybean, maize, tobacco, peanut,  
carrot or oil seed rape plant.

19. A method of inducing production of gamma  
linolenic acid (GLA) in an organism deficient or lacking  
20 in GLA which comprises transforming said organism with the  
isolated nucleic acid of any one of Claims 1-3.

20. A method of inducing production of gamma  
linolenic acid (GLA) in an organism deficient or lacking  
25 in GLA which comprises transforming said organism with the  
vector of any one of Claims 4-8.

21. A method of inducing production of gamma  
linolenic acid (GLA) in an organism deficient or lacking  
30 in GLA and linoleic acid (LA) which comprises transforming  
said organism with an isolated nucleic acid encoding

35



1 borage  $\Delta 6$ -desaturase and an isolated nucleic acid encoding  
12-desaturase.

22. The method of Claim 21 wherein said  
5 isolated nucleic acid encoding  $\Delta 6$ -desaturase comprises  
nucleotides 44 to 1390 of SEQ. ID NO: 4.

23. A method of inducing production of  
octadecatetraeonic acid in an organism deficient or  
10 lacking in gamma linolenic acid which comprises  
transforming said organism with the isolated nucleic acid  
of any one of Claims 1-3.

24. A method of inducing production of  
15 octadecatetraeonic acid in an organism deficient or  
lacking in gamma linolenic acid which comprises  
transforming said organism with the vector of any one of  
Claims 4-8.

20 25. The method of Claim 23 or 24 wherein said  
organism is a bacterium, a fungus, a plant or an animal.

26. A method of producing a plant with improved  
chilling resistance which comprises:

25 (a) transforming a plant cell with the isolated  
nucleic acid of any one of Claims 1-3; and

(b) regenerating said plant with improved  
chilling resistance from said transformed plant cell.

30 27. A method of producing a plant with improved  
chilling resistance which comprises:

1           (a) transforming a plant cell with the vector of  
any one of Claims 4-8; and

          (b) regenerating said plant with improved  
chilling resistance from said transformed plant cell.

5

28. The method of Claim 26 or 27 wherein said  
plant is a sunflower, soybean, maize, tobacco, peanut,  
carrot or oil seed rape plant.

10

15

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35

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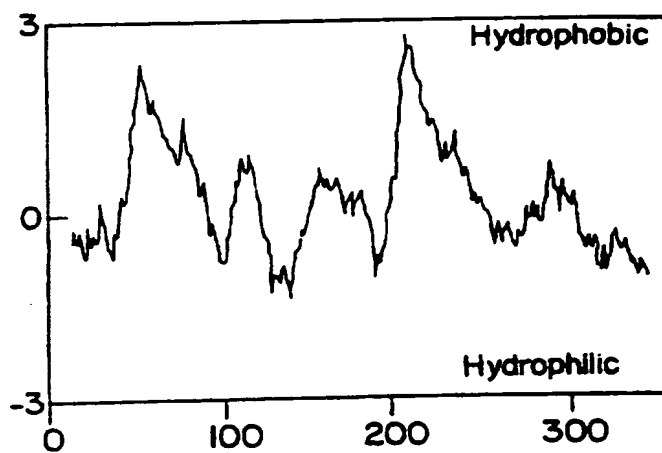
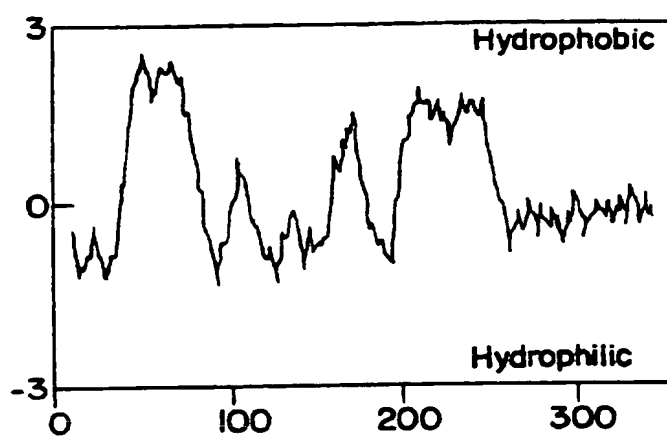


FIG. 2A

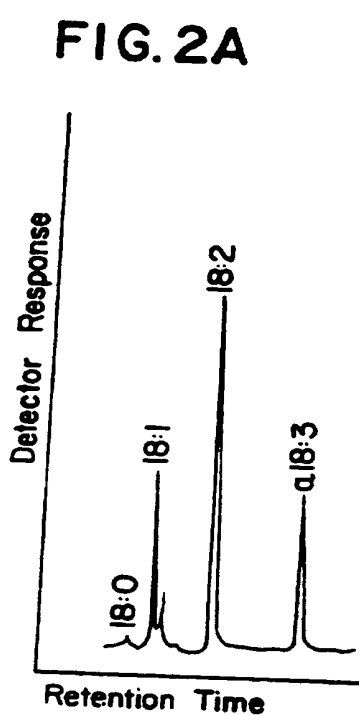


FIG. 2B

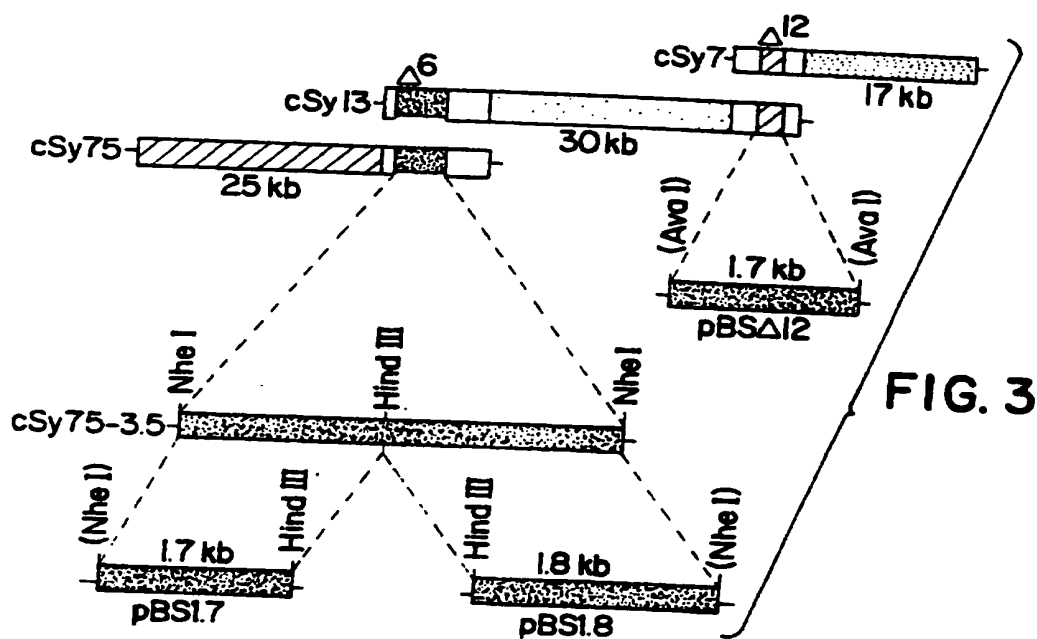
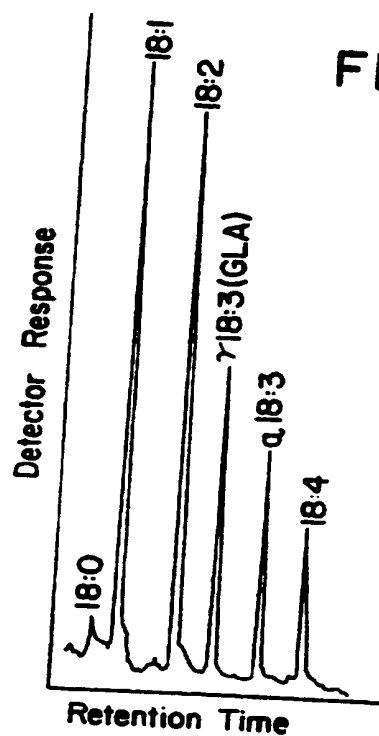


FIG. 3

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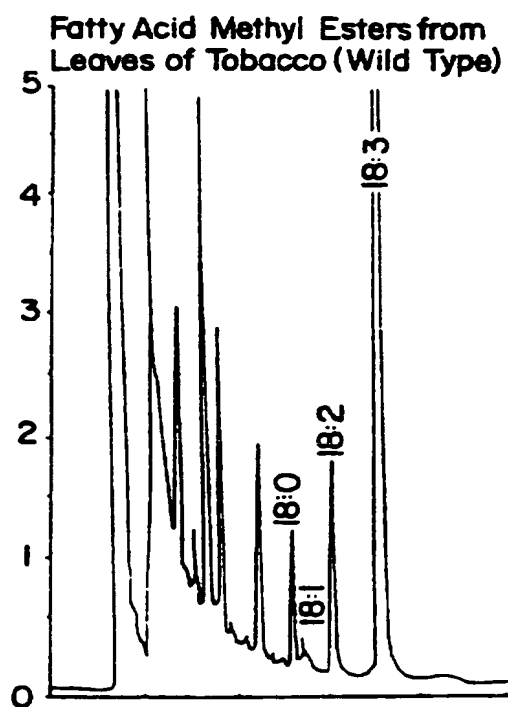


FIG. 4A

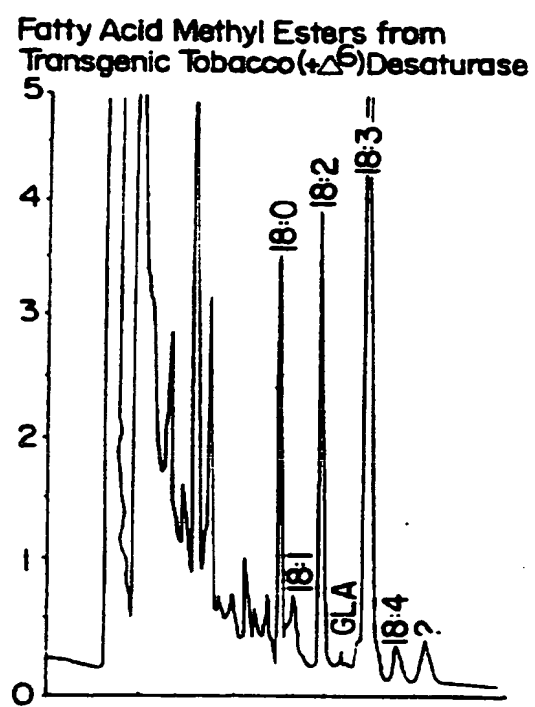


FIG. 4B

FIG. 5A

1 aatatctgcc taccctccca aagagagtag tcatTTTTtca  
 81 aactcaagaa ccacgataaa cccgagatc tatggatctc  
 161 gaccatcag gtggcagctt tcccttgaag agtcttgctg  
 241 ctctacatgg aagaatcttg ataatgtttt cactgggtat  
 321 atagggaagct tgtgtttgag ttttctaaaa tgggttttga  
 401 atagcaatgc tgtttgctat gagtgtttat ggggttttct  
 481 gatgggtttt ctttggattc agagtgtttg gattggacat  
 561 ataatgttat ggttattttt gctgcaaat gtctttcagg  
 641 cacattgcct gtaatatgct tgaatatgac cctgatttac  
 721 ttcactcacc tctcatttct atgagaaaaa gttgactttt  
 801 cattttaccc tattatgtgt cttggatgc tcaatatgta  
 881 tccctatcag ctcaggaact cttggatgc ctagtgttct  
 961 ggttgaaga attatgttg ttattgcaag ggaataattg  
 1041 ctccaagtgt ttatgttga aagcctaaa ggaataattg  
 1121 cttccttga tggattggtt tcatgttga ttgcaattcc  
 1201 ctttaggaaa atctgcctt acgtgacga gttatgcaag  
 1281 ccaatgaaat gacactcaga acattgagga acacagcatt  
 1361 gtatgggaag ctcttcacac tcatggttaa aattaccctt  
 1441 gtgtcttgc ttggttctac ttgttggagt cattgcaact  
 1521 gaggttttgc tttcatctcc attattgatg aataaggagt  
 1601 gaatgtactt tgtaccactg tgttttcagt tgaagctcat  
 1681 tattt  
 tcaatggctg ctcaaatcaa gaaatacatt acctcagatg 80  
 gattcaaggg aaagcctatg atgttttcga ttgggtgaaa 160  
 gtcaagaggt aactgatgca ttgtttgcat tccatcctgc 240  
 tatcttaaa atactctgt ttctgaggtt tctaaagatt 320  
 tgacaaaaaa ggtcatatta tgtttgcaac ttgtgtcttt 400  
 ttgtgaggg tgttttggtt catttggttt ctgggtgttt 480  
 gatgctggc attatatggt agtgtctgat tcaaggctta 560  
 aataagtatt ggttgggtga aatggaacca taatgcacat 640  
 aatatatacc attccttgtt gtgtcttcca agttttttg 720  
 gactctttat caagattctt tgtgaagttat caacattgga 800  
 tgtacaactt ctcataatgt tgttcttgtt tgcctaattg 880  
 cgatttggtt cccgttgcct gtttctctg aaccttctt 960  
 actggaatgc aacaagtcca gttctcttg aaccttctt 1040  
 gtttgagaaa caaacggatg ggacacttga catttcttct 1120  
 aattgagca tcatgttgtt cccaagatgc ctatgcaaa 1200  
 aaacataatt tgccttcaaa ttatgcatct tcttccaagg 1280  
 gcaggctagg gatataacca agccgtccc gaagaatttg 1360  
 agttcatgta ataatttgat attatgtatc tctatgttt 1440  
 tgtcttttat ggtttattag atgtttttta atatatatta 1520  
 tgcataattgt caattgttgt gctcaaatatc tgatatattt 1600  
 gtgtacttct atagactttg tttaaatggt tatgtcatgt 1680

FIG. 5B

1 MAAQIKKIYT SDELKNHDKP GDLWISIQGK AYDVSDWVKD  
 81 LKDYVSSEVS KDIRKLVFEF SKMGLYDKKG HIMFATLCFI  
 161 AGHYMVVSDS RLKFMGIFA ANCLSGISIG WKKWNHNAHH  
 241 SLRSREVSQ HWTYPIMCA ARLNMYVQSL IMLLTRNVVS  
 321 GMOQVQFSLN HFSSSVYVGK PKGNWFEKQ TDGTLDISCP  
 401 HNLPTYNASE SKANEMTLRT LRNTALQARD ITKPLPKNLV  
 HPGGSFPLKS LAGQEVTDAF VAFHPASTWK NLDKFFTGYY 80  
 AMLFAMSVYG VLFCEGLVH LFSGLMGFL WIOGHWIGHD 160  
 IACNSLEYDP DLQYIPFLV SSKFFGSLTS HFYEKRLTFD 240  
 YRAQELLGCL VFSIMYPLL SCLPNWGERI MFVIASLSVT 320  
 PMDWFHGG QFOIHHLP KMPRCNLRKI SPYVIELCKK 400  
 WEALHTHG  
 448



FIG. 7

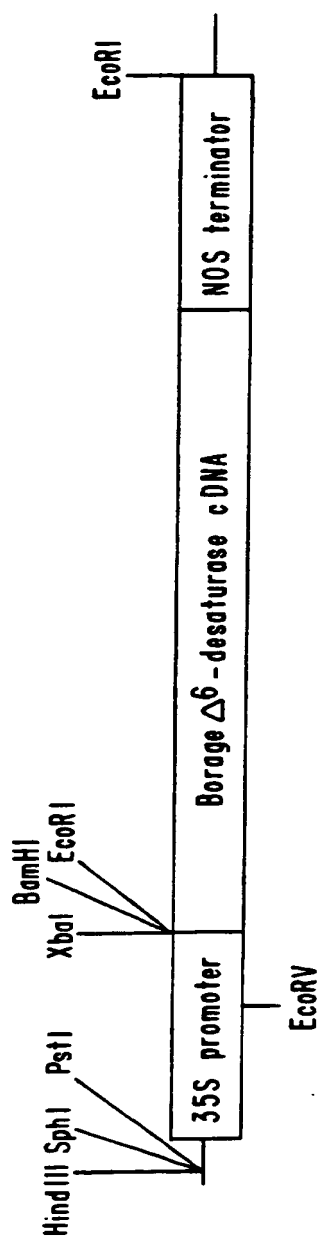




FIG. 8A

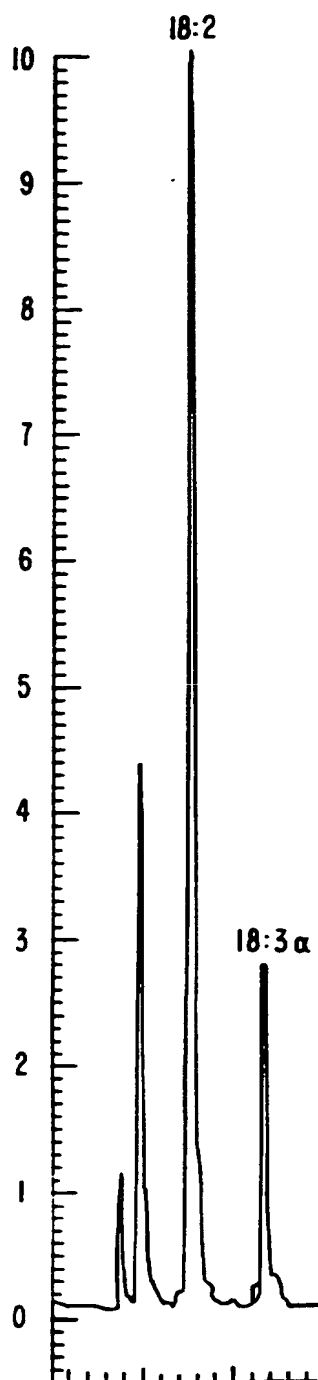


FIG. 8B

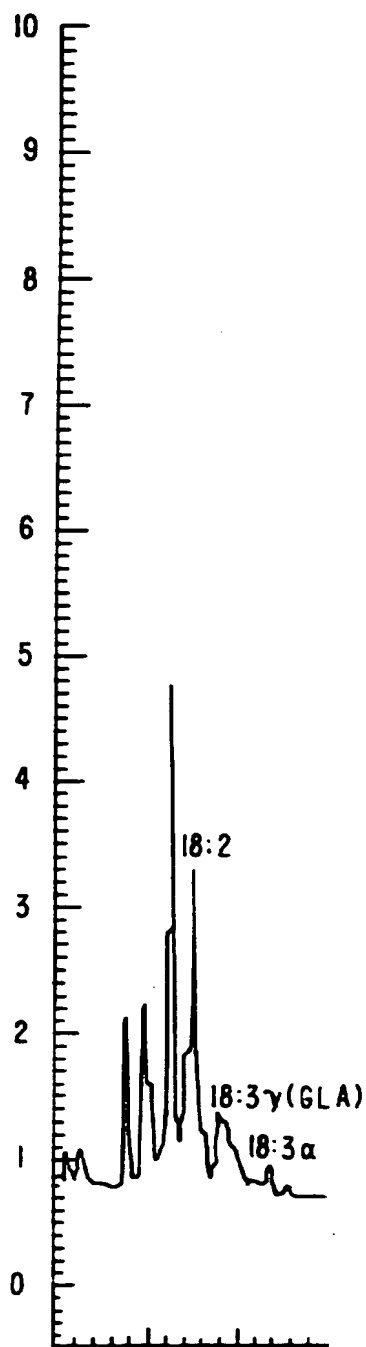
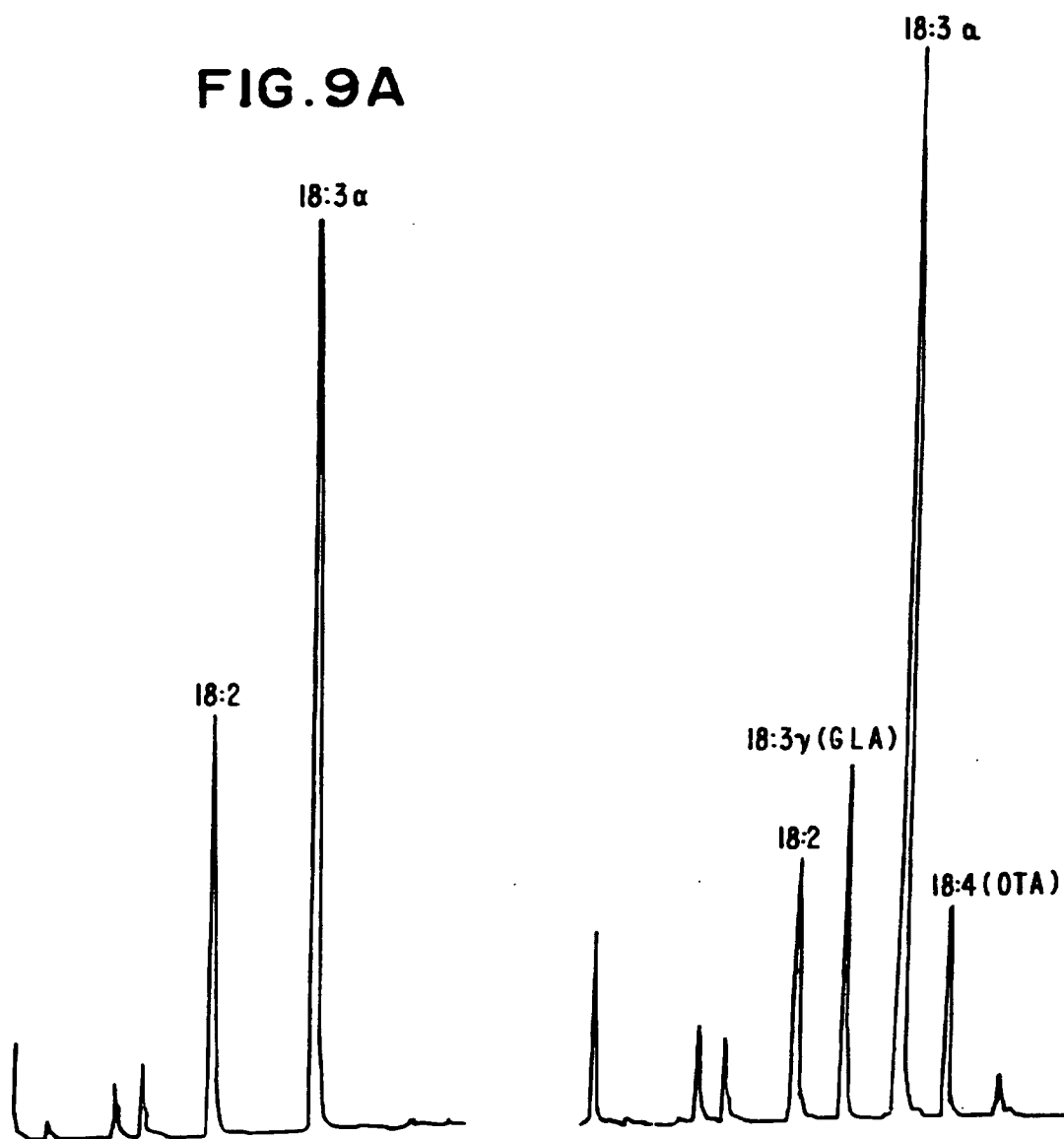


FIG. 9B



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FIG. IOA

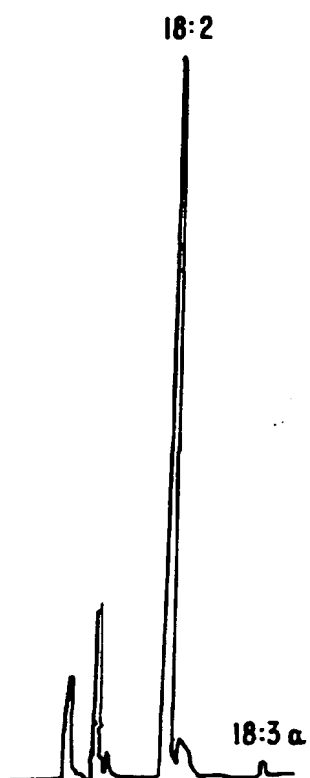
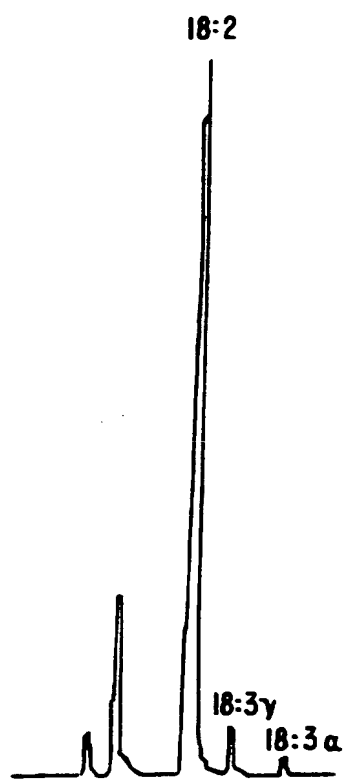


FIG. IOB



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## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>6</sup> :</b> <b>C12N 15/53, 15/82, A01H 5/00</b>	<b>A3</b>	<b>(11) International Publication Number:</b> <b>WO 96/21022</b> <b>(43) International Publication Date:</b> 11 July 1996 (11.07.96)
<b>(21) International Application Number:</b> PCT/IB95/01167 <b>(22) International Filing Date:</b> 28 December 1995 (28.12.95)  <b>(30) Priority Data:</b> 08/366,779 30 December 1994 (30.12.94) US  <b>(71) Applicant:</b> RHONE-POULENC AGROCHIMIE [FR/FR]; 14-20, rue Pierre-Baizet, F-69263 Lyon (FR).  <b>(72) Inventors:</b> THOMAS, Terry, L.; 3004 Normand, College Station, TX 77845 (US). REDDY, Avutu, S.; 3902 E. 29th Street #G11, Bryan, TX 77802 (US). NUCCIO, Michael; P.O. Box 553, College Station, TX 77841 (US). NUNBERG, Andrew, N.; 2804 B. Sprucewood Street, Bryan, TX 77801 (US). FREYSSINET, Georges, L.; 21, rue de Nervieux, F-69450 Saint-Cyr-au-Mont-d'Or (FR).  <b>(74) Agent:</b> MITSCHERLICH & PARTNER; Sonnenstrasse 33, D-80331 München (DE).		<b>(81) Designated States:</b> AU, BR, CA, CN, JP, RO, RU, UA, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).  <b>Published</b> <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>  <b>(88) Date of publication of the international search report:</b> 12 September 1996 (12.09.96)

**(54) Title:** PRODUCTION OF GAMMA LINOLENIC ACID BY A  $\Delta 6$ -DESATURASE**(57) Abstract**

Linoleic acid is converted into  $\gamma$ -linolenic acid by the enzyme  $\Delta 6$ -desaturase. The present invention is directed to isolated nucleic acids comprising the  $\Delta 6$ -desaturase gene. More particularly, the isolated nucleic acid comprises the promoter, coding region and termination regions of the  $\Delta 6$ -desaturase gene. The present invention provides recombinant constructions comprising the  $\Delta 6$ -desaturase coding region in functional combination with heterologous regulatory sequences. The nucleic acids and recombinant constructions of the instant invention are useful in the production of GLA in transgenic organisms.

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## INTERNATIONAL SEARCH REPORT

International Application No.

PLI/IB 95/01167

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/53 C12N15/82 A01H5/00

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N A01H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	KADER, J.-C. AND P. MAZLIAK (ED.). PLANT LIPID METABOLISM; 11TH INTERNATIONAL MEETING ON PLANT LIPIDS, PARIS, FRANCE, JUNE 26-JULY 1, 1994. XX+588P. KLUWER ACADEMIC PUBLISHERS: DORDRECHT, NETHERLANDS; NORWELL, MASSACHUSETTS, USA. 0 (0). 1995. 509-511. ISBN: 0-7923-3250-4, XP000569979 GALLE A-M, ET AL.: "Solubilization of DELTA-12- and DELTA-6-desaturases from seeds of borage microsomes." see the whole document --- -/-	1-28



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Date of the actual completion of the international search

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Date of mailing of the international search report

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## INTERNATIONAL SEARCH REPORT

International Application No

PLT/IB 95/01167

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